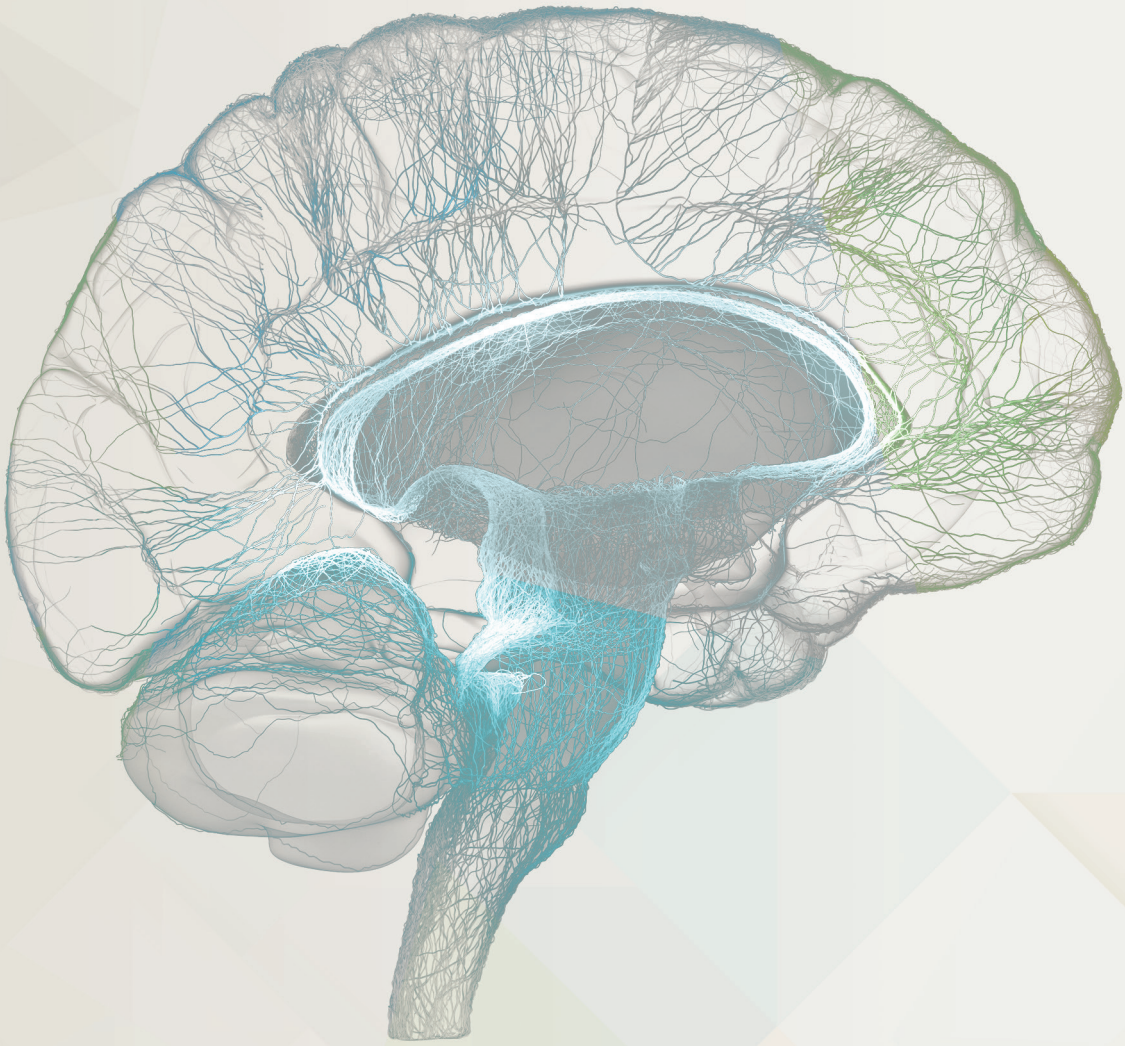
Relationships between cognitive deficits, central sensitization, and structural brain alterations in patients with chronic idiopathic neck pain, chronic whiplash associated disorders and fibromyalgia

Unravelling differences in underlying mechanisms

Iris Coppieters



Relationships between cognitive deficits, central sensitization, and structural brain alterations in patients with chronic idiopathic neck pain, chronic whiplash associated disorders and fibromyalgia

Unravelling differences in underlying mechanisms

Iris Coppieters

Thesis submitted in fulfillment of the requirements for the degree of Doctor in Health Sciences

Ghent University, 2017

Department of Rehabilitation Sciences and Physiotherapy
Faculty of Medicine and Health Sciences

Cover design and layout by In Zicht Grafisch Ontwerp, www.promotie-inzicht.nl
Printed by Ipskamp Printing, Enschede

200 YEARS GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

Promotor

Prof. dr. Mira Meeus	Ghent University, Ghent, Belgium University of Antwerp, Antwerp, Belgium

Co-promotor

Prof. dr. Barbara Cagnie	Ghent University, Ghent, Belgium

Supervisory Committee

Prof. dr. Karen Caeyenberghs	Australian Catholic University, Melbourne, Australia
Prof. dr. Lieven Danneels	Ghent University, Ghent, Belgium

Examination Board

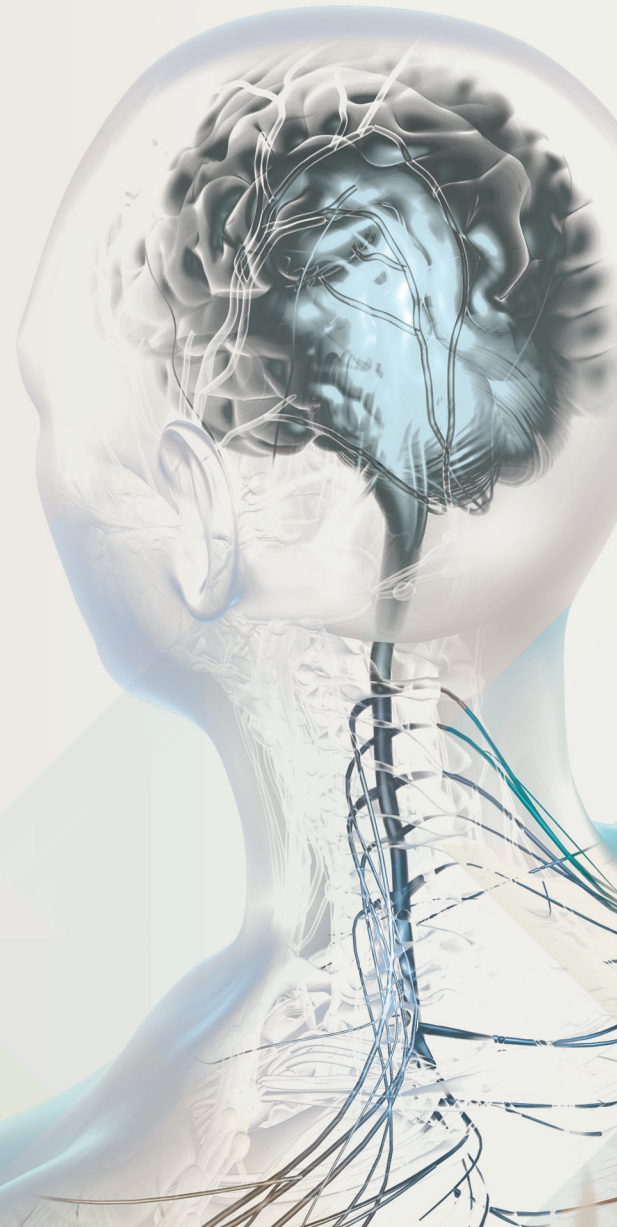
Prof. dr. Patrick Calders (chair)	Ghent University, Ghent, Belgium
Prof. dr. Birgit Castelein	Ghent University, Ghent, Belgium
dr. Roma Siugzdaite	Ghent University, Ghent, Belgium
Dr. Kristine Oostra	Ghent University Hospital, Ghent, Belgium
Prof. dr. Willem De Hertogh	University of Antwerp, Antwerp, Belgium
dr. Wendy Scholten-Peeters	Amsterdam University, Amsterdam, The Netherlands
dr. Inge Timmers	Maastricht University, Maastricht, The Netherlands

"Any man could, if he were so inclined, be the sculptor of his own brain."
- Santiago Ramón y Cajal

Table of contents

General Introduction

"One of the principle qualities of pain is that it demands an explanation."
- Anne Carson



The general introduction will first describe the study population included in this dissertation comprising patients with chronic idiopathic neck pain (INP), chronic whiplash associated disorders (WAD), and fibromyalgia (FM). Next, the neurophysiology of acute and chronic pain will be explained focusing on central sensitization (CS) and brain alterations in chronic musculoskeletal pain conditions. Also, the role of cognitive problems in patients with chronic musculoskeletal pain will be outlined. Finally, the last part of the introduction will describe the aims and outline of this dissertation.

1. Study population

Chronic neck pain is one of the most prevalent musculoskeletal pain conditions worldwide [1, 2]. In the general adult population, 30 to 50% will encounter an episode of neck pain in any given year [3], with a point prevalence of 10 to 20% [1]. Furthermore, neck pain tends to be a recurrent and persistent disorder as the majority of individuals with neck pain will not experience a complete resolution of their pain but will suffer from some degree of ongoing pain after their first episode [4, 5]. Unfortunately, the prevalence of chronic neck pain has been increasing, which results in growing socio-economic burden [6]. Disability-adjusted life-years due to neck pain increased globally from 23.9 million in 1990 to 33.6 million in 2010 [7]. Moreover, together with low back pain, neck pain is currently the leading global cause of disability [8]. Chronic neck pain is associated with high personal health expenditures and health care costs, often unexplained symptoms, frequent medical and physiotherapy consultations, and poor conservative therapy outcome [9-12]. It negatively affects activities of daily living, social participation, and work productivity, which results in burden among patients and their care providers [13].

Chronic neck pain can be subdivided, based on its etiology, into three categories i.e. 1) specific neck pain, 2) trauma-induced or whiplash-induced neck pain, and 3) idiopathic non-traumatic neck pain. This dissertation focuses on chronic neck pain of *idiopathic* and *traumatic* nature, which will be described in detail in the following paragraphs. In recent years, the focus on determining a peripheral pathoanatomical diagnosis of chronic neck pain has shifted towards an approach which attempts to unravel the underlying mechanisms within a broader multifactorial pathophysiological perspective since it is recognized that in the majority of neck pain patients an apparent pathoanatomical cause may not be identified [14]. As a consequence, there is much heterogeneity in clinical presentation and treatment response between and within the above mentioned two categories, thereby indicating differences in contributing mechanisms [15].

1.1. Chronic idiopathic neck pain

Chronic INP is characterized by persistent neck pain lasting more than three months, without the presence of a specific cause such as trauma, cervical hernias with clinical

symptoms or radiculopathy. No clear anatomical pathology can be demonstrated and radiological imaging findings are poorly related to patient's clinical symptoms [5].

In the absence of a specific pathoanatomical etiology, researchers have started to investigate possible underlying mechanisms of pain persistence in chronic INP. This multi-dimensional research has focused on sensorimotor control as well as articular, myofascial, and psychosocial dysfunctions [16-23]. To some extent, each of these dysfunctions could contribute to the persistent pain in chronic INP patients, yet it seems that subgroups are present within this heterogeneous condition [16-22]. Consequently, a paucity of studies have examined the role of pain mechanisms including research with respect to the presence of sensory hypoesthesia [22] and disturbed central pain processing [24]. Most studies could not reveal evidence for CS in the chronic INP population. However, few studies did find some evidence for distant hyperalgesia in chronic INP, resulting in an inconclusive message.

1.2. Chronic whiplash associated disorders

A whiplash injury usually originates from a rear-end motor vehicle crash and is caused by acceleration-deceleration forces acting on the neck, head, and torso [25, 26]. To date, the mechanism of whiplash injury is advocated to result from the inertial response of the body, which causes displacement of the neck and head without being exposed to direct impact [26]. Specifically, during a rear-end impact, the torso is rapidly carried forward resulting in thoracic and cervical spine straightening. This movement subsequently leads to a transitory 'S-shaped' cervical curvature, forcing the cervical spine into upper segmental flexion and lower segmental extension. As a result of this non-physiological motion it is proposed that energy is stored in the elastic cervical spine components. During the second phase of this motion, all cervical segmental levels are extended, followed by an abrupt energy release and subsequent forward thrust of the neck and the head. The described whiplash injury mechanism is illustrated in **figure 1**.

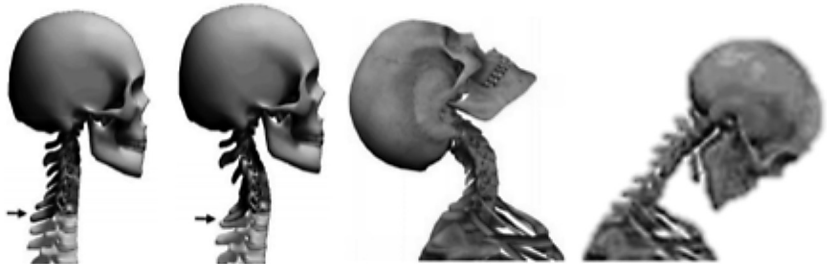


Figure 1 Mechanism of whiplash injury during a rear-end motor vehicle collision. Adapted from [26].

Lesions may occur to any cervical structure (e.g. intervertebral discs, ligaments, facet joints, muscles, nerve tissues) as a result of an acute whiplash injury [26]. Due to the poor sensitivity of current radiological imaging techniques these proposed lesions remain poorly identified. Nevertheless, it is also possible that in some people no lesions occur in the cervical spine and the surrounding tissues.

The trauma and the acceleration-deceleration mechanism of energy transfer to the neck and head may lead to the development of various clinical manifestations defined as WAD [27, 28], and defined as chronic WAD if the pain persists. Chronic WAD patients are characterized by trauma-induced chronic neck pain lasting more than three months [29]. By three months after the traumatic event, approximately one-third of whiplash patients will have developed persistent low levels of pain and related disability, one-third will have developed persistent high levels of pain and related disability, and one-third will have recovered from their initial pain and disability [30-34].

The predominant symptom in individuals with chronic WAD is neck pain, which can radiate to the head, shoulder, arm, interscapular, thoracic and lumbar regions [28]. Apart from persistent pain, a variety of other associated complaints are often reported by chronic WAD patients including numbness and tingling, concentration and memory problems, psychosocial deficits, post-traumatic stress, fatigue and sleep disturbances, headache, dizziness, and reduced quality of life [28, 35-41]. The wide range of debilitating symptoms and only partially elucidated underlying mechanisms in chronic WAD contribute to the low treatment effects in this prevalent and complex condition [9, 29].

The variable nature of symptoms encountered following a whiplash injury has led to a classification system in order to guide both research and treatment decision-making. In 1995, the Quebec Task Force (QTF) developed the QTF classification of WAD based on the type and severity of symptoms short after the traumatic event [39]. The QTF classified WAD patients into four grades ranging from zero (no neck pain complaints or physical signs) to grade four (neck pain complaints and fracture or dislocation). Since WAD grade II is the most common subgroup of the QTF classifications and covers a very broad range of signs and symptoms, the WAD II patient group was further divided into different categories [28, 42], encompassing physical, psychological, and physiological mechanisms [28, 43-45]. An improved classification system accounting for these prognostic factors was proposed by Sterling in 2004 [28] (**table 1**).

The research quest to uncover the pathophysiological mechanisms contributing to the persistent complaints of chronic WAD has increased our knowledge substantially, and has revealed motor, sensorimotor, psychological-affective, cognitive, and central pain processing dysfunctions [25, 26]. To date, a growing body of evidence supports the presence of CS as predominant pain mechanism contributing to the persistent symptoms in a large group of chronic WAD patients [35, 46-48]. Yet, many questions remain unresolved.

Table 1 Modified Quebec Task Force classification.

Proposed classification grade	Physical and psychological impairments present
WAD 0	No complaint about neck pain No physical signs
WAD I	Neck complaint of pain, stiffness, or tenderness only No physical signs
WAD II A	Neck pain Motor impairment - Decreased ROM
	- Altered muscle recruitment patterns (CCFT) Sensory impairment
	- Local cervical mechanical hyperalgesia
WAD II B	Neck pain Motor impairment - Decreased ROM
	- Altered muscle recruitment patterns (CCFT) Sensory impairment
	- Local cervical mechanical hyperalgesia
	Psychological impairment
	- Elevated psychological distress (GHQ-28, TSK)
WAD II C	Neck pain Motor impairment - Decreased ROM
	- Altered muscle recruitment patterns (CCFT) - Increased JPE
	Sensory impairment - Local cervical mechanical hyperalgesia
	- Generalized sensory hypersensitivity (mechanical, thermal, BPPT)
	- Some may show SNS disturbances
	Psychological impairment
	- Psychological distress (GHQ-28, TSK)
	- Symptoms of acute posttraumatic stress (IES)
WAD III	Neck pain Motor impairment - Decreased ROM
	- Altered muscle recruitment patterns (CCFT)
	- Increased JPE

Table 1 Modified Quebec Task Force classification.

Proposed classification grade	Physical and psychological impairments present
	Sensory impairment
	- Local cervical mechanical hyperalgesia
	- Generalized sensory hypersensitivity (mechanical, thermal, BPPT)
	- Some may show SNS disturbances
	Psychological impairment
	- Psychological distress (GHQ-28, TSK)
	- Elevated levels of acute posttraumatic stress (IES)
	Neurological signs of conduction loss including:
	- Decreased or absent deep tendon reflexes
	- Muscle weakness
	- Sensory deficits
WAD IV	Fracture or dislocation

Adapted from Sterling [28]. Abbreviations: WAD= whiplash associated disorders, ROM= range of motion, CCFT= craniocervical flexion test, GHQ-28= General Health Questionnaire-28, TSK= tampa scale for kinesiophobia, JPE= joint position error, BPPT= brachial plexus provocation test, IES= impact of events scale, SNS= sympathetic nervous system.

1.3. Fibromyalgia

FM is a common chronic musculoskeletal pain condition that depending on the diagnostic criteria used, affects between 1.2 and 5.4% in the general population [49], and between 2.4 and 6.8% in women [50]. According to the 1990, 2010, and modified 2010 American College of Rheumatology (ACR) criteria the ratio of females to males was 14:1, 5:1, and 2:1, respectively [49].

Chronic widespread musculoskeletal pain is the core feature of FM, however, most patients additionally report a wide range of co-occurring symptoms such as cognitive deficits including concentration and memory problems, headache, fatigue, and sleep disturbances [51-53]. Furthermore, FM patients often report anxiety and depression and present diminished quality of life [54]. Noteworthy, most of the neurotransmitters that affect pain transmission also affect mood, memory, sleep, and fatigue [55].

FM is a heterogeneous condition and multiple potential etiologies have been reported such as the influence of chronic stress, altered immune function, psychological distress, and CS [56]. Today, compelling evidence indicates that the pathophysiological hallmark of FM is a sensitized central nervous system (CNS) [55]. FM often co-occurs with

other conditions demonstrated to have a similar underlying pathophysiology such as tension-type headache, irritable bowel syndrome, temporomandibular disorders, chronic fatigue syndrome, and sometimes chronic WAD.

In 1990, the ACR developed criteria for the classification of FM [57]. These criteria required the presence of widespread pain for at least three months. In addition, tenderness on pressure, as determined by tender point examination, had to be present in at least 11 of 18 specified sites. Because criticism developed to these 1990 criteria, the ACR published new criteria for clinical diagnosis of FM that are suitable for use in primary and specialty care, do not require tender point examination, and take into account key co-occurring symptoms in FM [58]. To satisfy these diagnostic criteria for FM, the following three conditions have to be met: (1) a widespread pain index (WPI) score of ≥ 7 and symptom severity (SS) scale score of ≥ 5, or a WPI score between 3-6 and SS scale score of ≥ 9; (2) the symptoms have been present at a similar level for at least three months; and (3) the patient does not have a disorder that would otherwise explain the pain. The WPI is determined by noting the number of body regions in which the patient experienced pain over the last week. The score of the SS scale is the sum of the severity of three symptoms (i.e. fatigue, waking unrefreshed, and cognitive symptoms) plus the severity of somatic symptoms in general. In 2016, the ACR published an update of these 2010 criteria [59] and some modifications were made.

1.4. The continuum of chronic musculoskeletal pain

The three chronic pain conditions studied in the present thesis are all categorized as chronic musculoskeletal pain disorders. Based on the literature and clinical experience, we can infer that chronic INP, chronic WAD, and FM show overlapping symptoms and some similar mechanisms, however, they can also differ from each other in symptomatology, and hence in underlying pathophysiological mechanisms.

One of the possible reasons for observed differences in clinical presentation in these chronic musculoskeletal pain patients may be the fact that different **predominant pain mechanisms** can be present, being nociceptive pain, neuropathic pain, or CS pain [60-63]. Nociceptive pain is defined as pain arising from damage to non-neural tissue and is attributable to the activation of peripheral terminals of primary afferent neurons in response to noxious stimuli [63]. Clinically, nociceptive pain is recognized as pain being proportional to peripheral nociceptive input [61]. Neuropathic pain is defined as pain caused by a primary lesion or disease of the somatosensory nervous system [64]. As last, CS is defined as "an amplification of neural signaling within the CNS that elicits pain hypersensitivity" [65]. The clinical picture of the patient can be dominated by one of these pain mechanisms, however the presence of for example predominant neuropathic pain does not exclude the co-existence of CS pain. The awareness is growing that recognition of these pain mechanisms should be part of our clinical reasoning when assessing and treating individuals with chronic pain [60, 66, 67].

Extensive evidence supports the presence of CS as predominant pain mechanism contributing to pain persistence and associated symptoms in the majority of FM patients [56, 65, 68, 69], and in a large group of chronic WAD patients [35, 46-48]. On the contrary, based on a systematic review, CS is not a characteristic feature of chronic INP at group level, nevertheless CS can be present in some chronic INP patients, underpinning the possibility that subgroups exist within the chronic INP population [24].

It can be hypothesized that overlapping but also different underlying mechanisms exist between patients with chronic INP, patients with chronic WAD, and patients with FM. Furthermore, significant relationships between various symptoms and mechanisms are hypothesized (**fig. 2**).

Chronic INP, chronic WAD and FM are heterogeneous conditions possibly with patients falling along a continuum with at one end a purely peripherally driven (e.g. nociceptive) painful condition and at the other end of the continuum a pain condition that is purely centrally driven (CS) [56] (**fig. 3**). Furthermore, as proposed by Nielsen and Henriksson, FM may be the far end of a continuum that starts with chronic localized musculoskeletal pain and ends with widespread chronic disabling pain [70]. This way, the chronic INP group

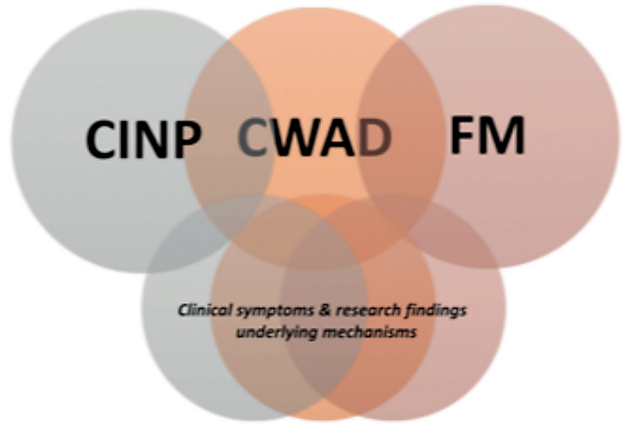


Figure 2 Presentation of the study population included in this dissertation with an illustration of the hypothesized overlap and differences between the three conditions with respect to clinical symptoms, research findings, and underlying mechanisms. The lower circles present the hypothesized relationships between different symptoms, research findings, and underlying mechanisms within each study group.

may be situated somewhere at the beginning of the continuum, followed by the chronic WAD group, and the FM group may be the far end of the chronic musculoskeletal pain continuum. Various individuals with chronic musculoskeletal pain may be floating at various points in this continuum [56], and thus some may have stronger peripheral than central components, some may have mixed peripheral and central components, and others may have stronger central components, and increasing severity and complexity of associated symptoms and psychosocial problems.

The following parts of the introduction will provide detailed information concerning the neurophysiology of acute and chronic pain, focusing on CS and reorganization of the brain in chronic musculoskeletal pain.

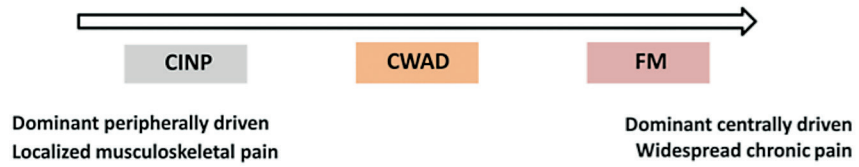


Figure 3 Continuum of chronic musculoskeletal pain.

2. Neurophysiology of acute pain

In 1979, the International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [71]. Since then, our understanding of pain has substantially increased, uncovering the biopsychosocial nature of pain. Accordingly, the following updated definition of pain was proposed in 2016 "pain is a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components" [72].

2.1. Nociception

The nerve cell endings that initiate the sensation of pain are called nociceptors (noci is derived from the Latin nocere, "to hurt") [73]. Nociceptors arise from cell bodies in dorsal root ganglia that send one axon to the periphery (the primary afferent neuron) and one axon to the spinal cord and brainstem (the secondary afferent neuron). Mainly two types of pain receptors are activated by nociceptive input [73]. These include low-threshold

nociceptors that are connected to fast conducting Aδ myelinated axons, and high-threshold nociceptors that convey impulses in slow unmyelinated axons or C-fibers. Finally, the stimuli are conveyed to the thalamus from where they are send to the cortex. The thalamus, as relay station, receives information from multiple surrounding brain regions with respect to the presumed threat and context of the stimuli. The nociceptive stimuli are perceived as pain only when they arrive at the cortex. Many neurotransmitters (i.e. glutamate, substance P) are able to modulate the postsynaptic responses via transmission to and from supraspinal sites (e.g. anterior cingulate and insular cortex) via the ascending and descending pain pathways [68, 74, 75].

2.2. Peripheral sensitization

The nervous system is highly plastic, and even with acute pain it undergoes some changes. When tissues are damaged and pain persists for a few days, this is the result of adaptations of unimodal nociceptors and enhanced responsiveness of polymodal nociceptive endings. More specifically, the nociceptor peripheral terminals become 'sensitized' after injury, by reducing their threshold, only within the site of injury where the terminal was exposed to products of tissue damage and inflammation [73, 76], causing the zone of primary or *local hyperalgesia* [77, 78] (**fig. 4**). Local hyperalgesia involves increased sensitivity to noxious stimulation at the local site of injury. The underlying process is defined as *peripheral sensitization* of nociceptors, and represents a protective action by the human body in order to prevent further use of damaged structures and consequent further damage of surrounding tissues.

For example, during an acute whiplash injury, the facet joints, which are abundantly innervated with Aδ- and C-fibers may become peripherally sensitized by capsular stretching, local pressure changes, and release of pro-inflammatory agents [26]. Furthermore, because of their anatomical location, the cervical dorsal root ganglia and nerve roots are vulnerable to injury during rapid acceleration-deceleration of the neck. In FM patients e.g., changes in intramuscular microcirculation could lead to ischemic muscle pain and peripheral sensitization [70] or in chronic INP patients active cervical myofascial trigger points could induce peripheral sensitization of nociceptors [79].

2.2.1. Primary or local hyperalgesia

The IASP defines hyperalgesia as "increased pain from a stimulus that normally provokes pain" [80]. Two types of hyperalgesia exist: primary or local hyperalgesia, and secondary or distant hyperalgesia which will be described later on. Local hyperalgesia can be assessed by measuring pain thresholds for mechanical, thermal, or electrical stimuli at the symptomatic regions. Accordingly, decreased pain thresholds at the primary painful region may represent local hyperalgesia and underlying peripheral sensitization, which has been demonstrated in the trapezius muscle and the neck in both chronic INP and chronic WAD patients compared to healthy controls [24, 46].

General Introduction

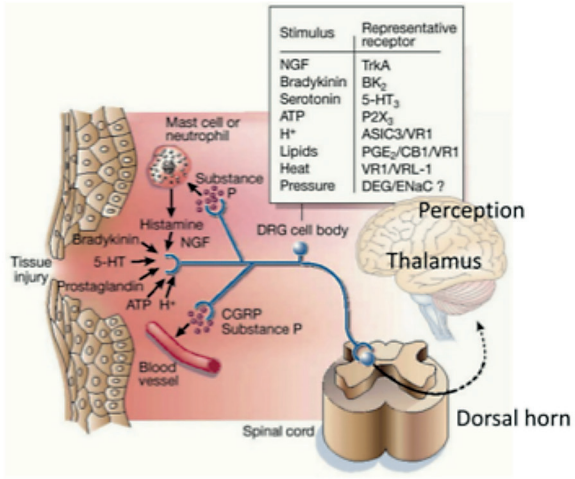


Figure 4 Mechanism of peripheral sensitization and nociceptive transmission to the dorsal horn and subsequently the brain. Adapted from [76].

3. Neurophysiology of chronic pain

Chronic pain is defined as prolonged and persistent pain of at least three months in duration [71, 81]. The IASP defines chronic pain as "pain without apparent biological value that has persisted beyond the normal tissue healing time (usually taken to be three months)" [82]. Prevalence estimates of chronic pain in the general adult population range from 10% to 55% with a higher prevalence among women [82].

3.1. Central sensitization

The pain mechanism that is crucially involved in the pathophysiology of numerous chronic pain states is CS [65]. CS is defined as "an increased responsiveness of nociceptive neurons in the CNS to normal or subthreshold afferent input" [83] or as "an amplification of neural signaling within the CNS that elicits pain hypersensitivity" [65].

The exact mechanisms by which the CNS becomes sensitized currently remain unknown, however some contributing factors have been proposed, which will be succinctly explained. CS is an expression of structural, functional, and chemical changes in the properties of neurons in the somatosensory CNS, and reflects remarkable neuroplasticity occurring at different levels of the CNS (primary sensory and dorsal horn neurons, ascending and descending pain pathways, the brain) [65, 70, 83, 84]. Neuronal plasticity, possible throughout life, refers to the ability of the CNS to reorganize itself in response to experience and thereby adapt, but also maladapt its structure and function [85]. Hence, neuroplasticity can be adaptive for example in the case of learning and memory, however, in the pathophysiology of chronic pain including CS pain, neuroplasticity is maladaptive.

In contrast to peripheral sensitization, which generally requires ongoing peripheral noxious stimulation for its maintenance, CS produces pain hypersensitivity in non-inflamated or non-damaged tissues [83]. Spontaneous activity in dorsal horn neurons and enlargement of their receptive fields, reduction in the activation threshold by peripheral stimuli and increased response to suprathreshold stimuli, as well as increased synaptic strength in dorsal horn neurons, among others are present in CS [65, 83]. Especially, the wide dynamic range neurons in the dorsal horn, change in structure, function, and biochemistry [70]. Together, these changes in neuronal properties result in increased action potential output in response to previously subthreshold synaptic inputs. This way, CS represents an 'uncoupling' of the clear peripheral stimulus response relationship.

In addition, once CS is established, it remains highly plastic. Hence, any new peripheral injury may serve as a new source of afferent peripheral nociceptive input, which in turn sustains or aggravates CS [86]. Nonetheless, without new peripheral input, CS will not resolve easily and is also able to sustain the chronic symptoms of the patient because of the described neuronal changes. In addition, alterations in microglia, astrocytes, and gene transcription all may contribute to the maintenance of CS [65, 87-89].

To date, considerable evidence shows that changes in neural processing in the CNS contribute to widespread pain hypersensitivity in various chronic musculoskeletal pain disorders, presenting overlapping symptoms [65]. Accordingly, an important clinical implication is that the experience of pain, in for example patients with chronic WAD [35, 46-48] and FM [65, 68, 69] with CS, not necessarily reflects the presence of peripheral noxious stimulation but is rather the result of CNS alterations. Spinal cord hypersensitivity has for instance objectively been demonstrated by decreased nociceptive flexion reflex thresholds in chronic WAD and FM patients, which provides compelling evidence of neurophysiological overlap between both conditions [90]. Chronic pain is thus not a passive consequence of transmission of a defined peripheral input to a cortical pain region, but a reflection of *dynamic central neuroplasticity* (although maybe partially generated in the periphery) [84].

3.1.1. Secondary or distant hyperalgesia

Distant hyperalgesia involves increased sensitivity extending beyond the site of injury, and hence is believed to result from sensitization of central nociceptive signaling neurons [91]. Quantitative Sensory Testing (QST) has been recommended to investigate signs of CS such as distant hyperalgesia for a variety of stimuli (mechanical, thermal, electrical) [92]. Various QST tools have been developed to quantify different aspects of CS. Gold standards for examining CS are currently lacking and because numerous underlying mechanisms contribute to CS, it is advocated to assess a combination of QST measures (e.g. distant

hyperalgesia, endogenous pain inhibition, temporal summation (TS) as explained further on) in order to reveal the presence of CS.

Evidence is present to support distant hyperalgesia (i.e. decreased pain thresholds at non-injured distant regions compared to healthy persons) for mechanical, thermal (cold and heat) and electrical stimuli in patients with chronic WAD and FM [46, 48, 68]. Results of the scarce studies available investigating distant hyperalgesia in chronic INP are variable and inconclusive [24].

3.1.2. Allodynia

Another clinical and experimental sign of CS is distant allodynia, which is defined as pain to a stimulus that does normally not provoke pain, such as light pressure, touch, or brushing [80, 93] (**fig. 5 and 6**). Allodynia results from phenotypic changes in Aβ-fibers with low-threshold mechanoreceptors. It is demonstrated that Aβ-fibers obtain qualities that are similar to those of C-fibers. As a consequence, these fibers will also convey pain [94]. In case of CS, allodynia is the consequence of hyperexcitability of wide dynamic range neurons to input from cutaneous C-fiber stimuli [95]. Allodynia can be present at the local injured symptomatic area in case of peripheral sensitization, but when allodynia arises at distant non-injured regions this is an indirect sign of CS [70].

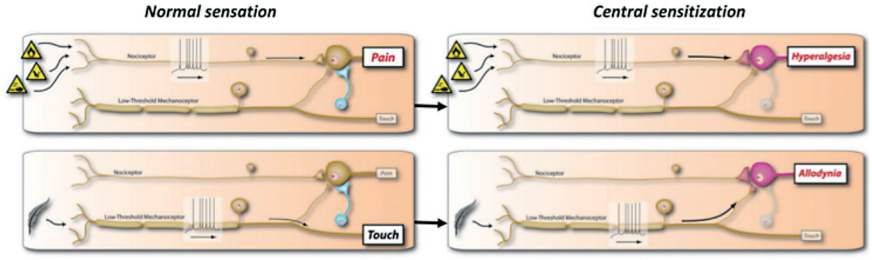


Figure 5 Illustration of normal sensation (left) and presentation of CS mechanisms manifesting as hyperalgesia and allodynia (right).

(Left) The somatosensory system is organized such that the highly specialized primary sensory neurons that encode stimuli with low intensity only activate the central pathways that lead to innocuous sensations, while high intensity stimuli that activate nociceptors only activate the central pathways that lead to pain and the two parallel pathways do not functionally cross. (Right) With the installation of CS in somatosensory pathways with increased synaptic efficacy and reduced inhibition, a central amplification occurs enhancing the pain response to noxious stimuli in amplitude, duration and spatial extent, while the strengthening of normally ineffective synapses recruits subliminal inputs such that inputs in low threshold sensory inputs can now also activate the pain circuit. The two parallel sensory pathways converge. Reprinted from Woolf [65].

3.1.3. Endogenous pain modulation

In general, *endogenous pain modulation* delineates the actions that the CNS can use to modulate pain. There are two major pain modulatory mechanisms including *pain facilitation* and *pain inhibition* [96]. In the following paragraphs, pain facilitation and pain inhibition will be described in detail.

3.1.4. Changes in ascending pathways or overactivation of the bottom-up system

Overactivation of the bottom-up system is one possible contributing mechanism of CS [69]. Wind-up and long-term potentiation (LTP) are two important mechanisms contributing to the increased efficiency of nociceptive signaling to the brain and will be explained in the following section. In particular, LTP is an important neurobiological source and contributor of CS.

3.1.4.1. Wind-up and temporal summation (TS) of second pain

Wind-up occurs after repeated low-frequency (0.33 – 5 Hz) C-fiber stimulation at constant intensity and represents a progressive increase in action potential firing of the second-order neuron over the course of the stimulus. In particular, wind-up depends on activation of nociceptor-specific neurons and wide dynamic range neurons in the dorsal horn of the spinal cord [75, 97]. TS of second pain is the perceptual correlate in humans of wind-up in the

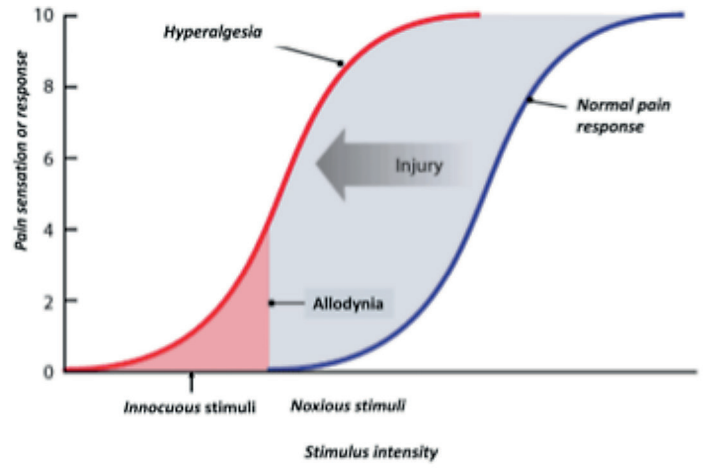


Figure 6 Hyperalgesia and allodynia.

The normal pain response as a function of stimulus intensity is depicted by the curve at the right, where even strong stimuli are not experienced as pain. However, an injury can shift the curve to the left. Then, noxious stimuli become more painful (hyperalgesia) and typically painless stimuli are experienced as pain (allodynia).

dorsal horn and entails increasing pain perception or pain ratings in response to repeated noxious stimuli of equal intensity and duration delivered at low frequencies [98, 99] (**fig. 7**). Second pain, which is more dull and strongly related to chronic pain states, is transmitted through unmyelinated C-fibers to dorsal horn nociceptive neurons. TS of second pain is caused by the repeated stimulation of these C-fibers at frequencies between 0.33 – 5 Hz, leading to progressively increasing electrical responses in the corresponding spinal cord (posterior horn) neurons [95, 100].

Noteworthy, wind-up only takes place in the dorsal horn of the spinal cord. It is a *short-lasting* phenomenon that enhances action potential firing of dorsal horn neurons to the first 10–30 stimuli i.e. during the first few seconds of an ongoing noxious stimulus [95]. Thereafter, the response reaches a plateau or may decline as the membrane potential returns to its normal resting level [83]. This electrophysiological phenomenon is also present in healthy pain-free persons, and thus constitutes a normal coding property of nociceptive spinal dorsal horn neurons, being not per se a sign of CS [95].

But in patients characterized by CS, wind-up is strengthened, which means stronger wind-up and lowering of the wind-up threshold frequency of a given neuron [83], indicating the presence of facilitation existing in spinal nociceptive pathways. Therefore, TS of second pain is widely used in pain research as an experimental paradigm to assess the latter CNS hyperexcitability [46, 101-103]. This research has demonstrated increased TS of second pain in patients with chronic WAD and FM [46, 69], but no clear evidence for increased TS is available in chronic INP patients [24].

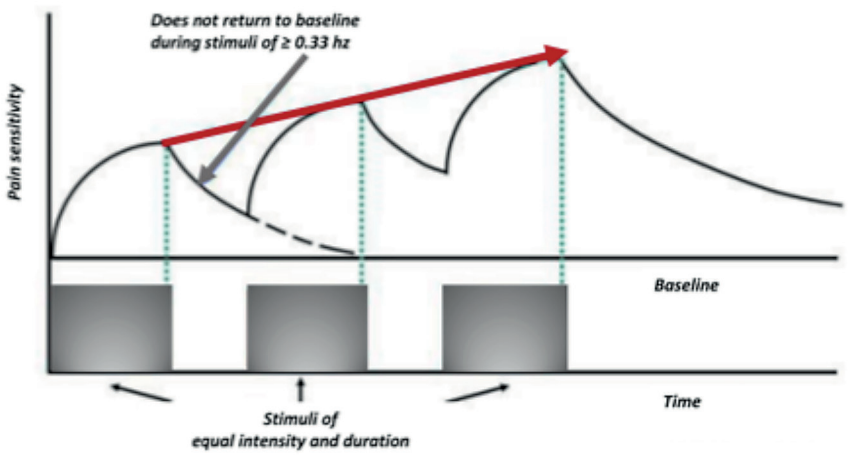


Figure 7 Illustration of temporal summation of second pain demonstrated by increasing pain sensitivity in response to repeated noxious stimuli of equal intensity and duration delivered at a frequency of 0.33 Hz or more.

3.1.4.2. Long-term potentiation (LTP) in the dorsal horn and the brain

In case of excessive accumulation of glutamate in the synaptic cleft between C-fibers and second-order neurons, due to persistent strong stimulation, more long-term changes may occur. Long-term synaptic plasticity has been identified in the spinal dorsal horn, in particular at C-fiber synapses, and in the brain [83, 104, 105]. After repetitive (0.5 – 5 Hz) synaptic stimulation, *long-lasting* increase in synaptic strengthening can be installed, known as LTP. LTP can manifest after repetitive stimulation in a short-time frame (e.g. seven successive days), defined as *early* LTP [83, 106] (**fig. 8**) encompassing a phosphorylation-dependent and transcription-independent phase resulting mainly from rapid changes in glutamate receptors and ion channel properties [84].

The *later*, longer-lasting LTP, which manifests after repetitive stimulation in a longer time frame (e.g. months) is transcription-dependent and drives synthesis of new proteins responsible for the longer-lasting form of CS observed in chronic pain conditions [83]. The synaptic strength can increase after repetitive stimulation because synapses in the CNS are plastic and can adapt through modifications in the presynaptic terminal (increases its capacity to produce and release neurotransmitters) and adaptations in the postsynaptic neuron (increases its capacity to bind neurotransmitters due to increased numbers of receptors at the postsynaptic cell membrane) [83, 104]. As a consequence, CNS neurons become more efficient in nociceptive transmission. Once LTP has been installed, identical presynaptic stimuli will now lead to a much powerful and longer-lasting response of the post-synaptic neuron. During the C-fibers transmitted stimuli, N-methyl-D-aspartate (NMDA) receptors of second-order neurons become activated. NMDA activation induces calcium entry into the dorsal horn neurons [107], which leads to nitric oxide (NO) synthesis [108]. NO can subsequently affect the nociceptor terminals and enhance the release of neuropeptides (substance P), therefore contributing to the maintenance of CS [109]. Substance P lowers the threshold of synaptic excitability, resulting in the unmasking of normally silent interspinal synapses and the sensitization of second-order neurons [110]. Furthermore, substance P can expand for long distances in the spinal cord and thereby sensitize dorsal horn neurons at a distance from the nociceptive input location. This results in an expansion of receptive fields and the activation of wide dynamic range neurons by non-nociceptive afferent impulses [75].

Moreover, in contrast to wind-up, persistent LTP does not require continuous neuronal activity from the periphery, and can have a duration of up to months or even longer [106]. LTP is a crucial mechanism contributing to CS [106], and is one of the mechanisms underpinning brain alterations associated with chronic pain [111]. This mechanism has been most thoroughly studied in the hippocampus and the amygdala, and is reported to be a neurobiological substrate for learning and memory, through synaptic strengthening and remodeling of neural networks [105, 111, 112]. A role in fear and pain-related cognitive emotional factors of LTP in the brain has also been established [106]. Research has furthermore put forward that LTP could induce the formation of the so-called 'pain memory' both in the

spinal cord and the brain in various chronic pain states, including those with CS [113, 114]. Hence, in the light of LTP, chronic CS pain can partially be understood as a learned mechanism that is now "marked" in the spinal cord and the brain, the so-called "*neural pain signature*" [115]. Research towards the molecular mechanisms of CS and LTP indicates that, although there are differences between the synaptic plasticity contributing to memory and pain, there are also striking similarities [105]. Preventing or 'erasing' LTP may serve as an important target to inhibit chronic pain in patients in the future [104].

3.1.5. Changes in descending pathways or alterations in the top-down system

The dorsal horn of the spinal cord is the location of the first synapse in pain pathways, and as such, offers a powerful target for the regulation of nociceptive transmission not only by local dorsal horn mechanisms (as described above) but also by supraspinal modulation [116]. Descending facilitatory and inhibitory neuronal pathways can influence the excitability of the spinal cord through this supraspinal modulation [117]. This descending control on spinal nociceptive processes arises from a number of supraspinal sites, for example the periaqueductal grey (PAG)-rostral ventromedial medulla (RVM) system [116], as well as the subnucleus reticularis dorsalis [118]. The PAG is interconnected with the hypothalamus and limbic forebrain regions including the amygdala, and projects to the RVM, which in turn sends its output to dorsal horn laminae involved in nociception. The subnucleus reticularis dorsalis communicates with the PAG and RVM, and projects directly to laminae of the dorsal horn including projection neurons which themselves project to the subnucleus reticularis dorsalis, thereby closing a reverberating loop. Mechanisms originating in the subnucleus reticularis dorsalis and in other supraspinal sites can mediate either descending facilitation or inhibition.

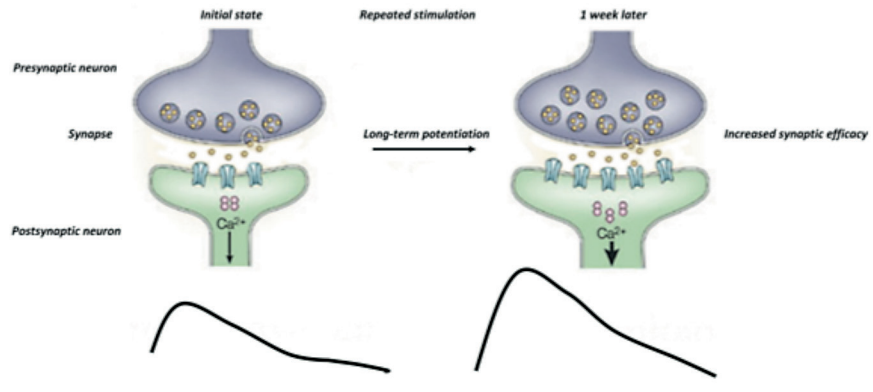


Figure 8 Mechanism of long-term potentiation in the dorsal horn of the spinal cord and the brain.

In healthy pain-free persons, a balance exists between activity in descending inhibitory and facilitatory pathways. In case of CS in chronic pain states, it seems that an imbalance of these pain modulatory mechanisms emerges, turning the balance in favor of facilitation and leading to sensitization of second-order neurons, hence increasing the gain of nociceptive processing [112, 116].

3.1.5.1 Increased activation of descending facilitatory pathways

Cognitive emotional sensitization refers to the capacity of a number of regions located in the forebrain to exert powerful influences on various brainstem nuclei, including the nuclei identified as the origin of descending facilitatory pathways [112, 119]. Specifically, anatomical connections have been demonstrated between forebrain regions and the RVM, both directly as well as via the PAG [112]. These regions include for example the anterior cingulate and insular cortices and subcortical hypothalamic and amygdalae nuclei. Connections between these limbic brain regions and descending facilitatory pathways thus play a neurophysiological role in cognitive emotional sensitization [120]. This yields the clinical important knowledge that the activity in descending pathways is not constant but can be modulated by the level of attention, motivation, stress, anxiety, maladaptive pain cognitions or psychological correlates such as pain hypervigilance, catastrophizing, pain-related fear and kinesiophobia, avoidance behaviors, among others [112, 119, 121]. Accordingly, a person's pain experience can be influenced by the interplay of these cognitive-emotional and psychosocial factors.

3.1.5.2 Dysfunctional activation of descending inhibitory pathways

The descending pain inhibitory system exerts its analgesic influence on the CNS through actions at the 'off-cells' in the RVM, and the release of neurotransmitters such as serotonin and noradrenaline [118]. Research suggests that disruption of one or more of the elements of the inhibitory system can result in the equivalent of CS [112]. A crucial function of the descending inhibitory pathway is to 'focus' the excitation of the dorsal horn neurons. The aim is to generate a more urgent, rapid, and localized pain signal by suppressing surrounding neuronal activity [84]. This role is attributed to the 'diffuse noxious inhibitory controls' phenomenon [122] which is now called 'conditioned pain modulation (CPM)' based on expert recommendations [123]. The term CPM was introduced in 2010 to describe the psychophysical paradigm to test diffuse noxious inhibitory controls [123, 124]. According to this mechanism, descending pathways effectively enhance the biologically valuable pain signal by reducing the level of irrelevant 'noise' in the system. Research has reported that adequate activation of pain inhibition by the CPM response also seems to be under control of brain regions such as the orbitofrontal cortex and the amygdala [125].

Inefficient endogenous pain inhibition and dysfunctional CPM activation have been demonstrated in patients with chronic WAD and FM [126-130]. Only one previous study has investigated CPM in patients with chronic INP, and found no evidence for dysfunctional CPM efficacy [131].

In addition to the 'pain inhibits pain CPM paradigm' it is recognized that also stress is an important factor that can exert complex modulatory influences on pain, and on descending pain inhibition and facilitation [132, 133]. In particular, stress can have a major impact on pain perception by either suppressing pain (stress-induced analgesia) [132] or exacerbating it (stress-induced hyperalgesia) [133].

The autonomic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis are primary responsible to respond adequately to stress [134] in life threatening situations but also in response to daily stressors. The acute short-term stress response is predominantly regulated by the sympathetic subdivision of the autonomic nervous system, whereas the later evoked and longer lasting stress response is mainly induced by the HPA-axis with releasing cortisol as most important stress hormone.

Many chronic pain patients display however a dysfunctional stress response [135]. Clinically, this dysfunctional stress response becomes clear when chronic pain patients report that they are no longer able to deal with daily physical and psychological stressors, which were no problem to handle before their chronic pain problem. Furthermore, patients report that a mild stressor can already worsen their pain complaints. The latter is remarkable because one of the main functions of the stress response systems is activation of endogenous top-down pain inhibition in a stressful situation [118]. As such, the stress hormones cortisol and noradrenaline are both pain inhibiting, and noradrenaline is an important neurotransmitter that contributes to pain inhibition exerted via the descending pain pathways [118]. At the level of the spinal cord, cortisol binds at the glucocorticoid receptors of the dorsal horn, where cortisol performs its pain dampening actions [136]. Noteworthy, the pain inhibiting functions of the stress systems are logical from an evolutionary survival based theory, however, when in chronic pain patients, daily stressors only worsen their pain severity and associated symptoms, this is clearly an indication that the stress response system in these patients has become dysfunctional [135].

Research has proposed four important mechanisms underlying the dysfunctional stress response in chronic pain patients such as patients with chronic WAD and FM.

- Decreased availability of inhibitory neurotransmitters such as Gamma-Aminobutyric acid and serotonin in the CNS including the brain [137, 138].
- Exhausted HPA-axis which results in decreased production of the stress hormone cortisol in response to a stressor [139-141].
- Stress activates in chronic pain patients the 'on-cells' and suppresses the 'off-cells' activity in the hypothalamus, which turns the balance in favor of pain facilitation instead of pain inhibition [142, 143].
- Decreased ability of the parasympathetic part of the autonomic nervous system to recover the body of chronic pain patients from a stressful situation [144, 145].

A dysfunctional stress response system has been demonstrated in patients with chronic WAD [139] and FM [140, 141, 145]. For example, dysfunctions of the HPA-axis have been found in both chronic pain conditions [139, 146, 147]. In whiplash patients, stress response systems seem to influence the development of chronic pain after a motor vehicle crash. Due to the traumatic event, post-traumatic stress can develop in patients with WAD, and it is indeed demonstrated that post-traumatic stress is present in a subgroup of chronic WAD patients [148]. Moreover, symptoms of post-traumatic stress in acute/subacute WAD patients is prognostic of poor recovery [149].

To conclude, the experience of chronic stress which results in dysfunctional stress response systems is another overlapping clinical feature within patients with chronic WAD and FM. In contrast, to the best of our knowledge no research or evidence exists supporting the presence of alterations in the stress response systems in patients with chronic INP. Moreover, the hypothesized relationship between stress and disturbed central pain modulation in chronic WAD and FM has not been clearly investigated.

3.2. Structural and functional reorganization of the brain in chronic musculoskeletal pain

"The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory." - Santiago Ramon y Cajal

Understanding the human brain is one of the most difficult challenges that scientists have faced in the past and it will probably remain one of the most complex challenges in the future. Nonetheless, what we know about the brain is increasing and changing at a breathtaking pace. Scientists long viewed the adult brain as a relatively static organ, but now it is clear that the brain is constantly changing its structural and functional organization as a function of experience, and hence is highly plastic [150].

Before we will elaborate on the role of brain plasticity and brain alterations in chronic musculoskeletal pain and associated symptoms, the following section will concisely describe the constitution of the human brain. Subsequently, we will succinctly run through the basic principles of MRI, T1-weighted structural MRI and Diffusion-Weighted Imaging (DWI) in order to provide the reader with sufficient theoretical background information on the different brain MRI techniques applied within this dissertation.

3.2.1. Human brain anatomy

The brain is parcellated into three main components namely the cerebrum, the cerebellum and the brainstem [151]. The cerebrum comprises the left and right cerebral hemispheres, the interbrain between the cerebrum and the brainstem termed the diencephalon, and the deep grey nuclei. The cerebral hemispheres are the largest compartments of the brain

and are interconnected by *white matter* (WM) fibers. The hemispheres are composed of outer *grey matter* (GM) termed the cerebral cortex and inner WM encompassing the deep grey nuclei (e.g. thalamus, amygdala, hippocampus) (**fig. 9**). The GM contains mainly nerve cell bodies, while the WM is made up predominantly of nerve fibers (axons). Cerebrospinal fluid surrounds the brain and acts as a cushion for absorbing the impact from light trauma, and maintains CNS homeostasis. In addition, within the brain there is a ventricular system, which contains four interconnected cerebral ventricles filled with cerebrospinal fluid. Furthermore, brain protection is provided by meninges surrounding the brain.

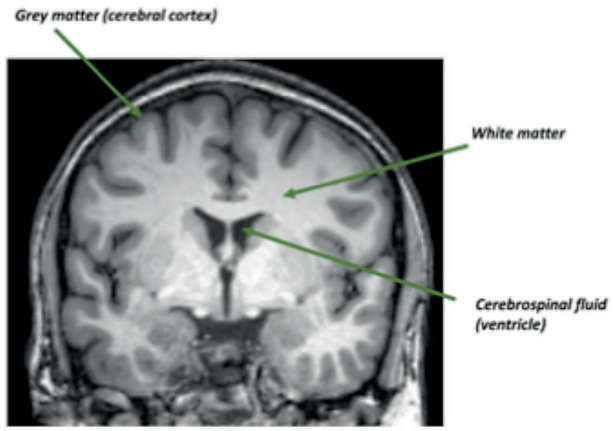


Figure 9 A T1-weighted Magnetic Resonance Image illustrating cortical grey matter, white matter and ventricles filled with cerebrospinal fluid.

3.2.2. Magnetic Resonance Imaging

"In physics, you don't have to go around making trouble for yourself, nature does it for you."
– Frank Wilczek

MRI is based on noninvasive radio frequency (RF) excitation of biological molecules with magnetic properties [152]. To generate MR images, the hydrogen nucleus (a single proton) of water (H_2O) is used. The hydrogen proton can be likened to the planet earth, spinning on its axis which creates an intrinsic angular momentum, with a north-south pole. In this respect the hydrogen atom ^1H can be represented as a magnetic moment vector that causes the nucleus to behave like a small bar magnet, similar to a compass-needle. Under normal circumstances, these hydrogen proton "bar magnets" spin in the body with their axes randomly aligned (**fig. 10**). The proton spins are randomly distributed due to thermal

agitation and the Brownian motion, which results in no net magnetization vector for the protons in the brain.

When protons are placed in an extern magnetic field B_0, e.g. when a person is placed in the MRI scanner (or the earth magnetic field in case of the compass-needle) they will align in the direction of this field. When a RF-pulse is applied to excite the protons (or tap the compass-needle), they will start oscillating with a constant frequency. This excitation is necessary to measure the signal, if you do not tap the compass-needle, the oscillation-frequency cannot be determined. In other words, when the body is placed in a strong magnetic field, such as an MRI scanner, the protons' axes all line up. This uniform alignment creates a magnetic vector oriented along the axis of the MRI scanner. When additional energy in the form of a radio wave is added to the magnetic field, the magnetic vector is deflected. The RF-pulse gives a transverse component to the magnetization of the vector of all protons. This is called resonance. When the RF source is switched off, the magnetic vector returns to its resting state, and this causes a radio wave to be emitted. The time needed to realign is the relaxation time.

The strength of the magnetic field can be altered electronically using a series of gradient electric coils, and by altering the local magnetic field by these small increments, different slices of the body will resonate as different frequencies are applied. Receiver coils are used around the body part in question for example the brain to act as aerials to improve the detection of the emitted signal.

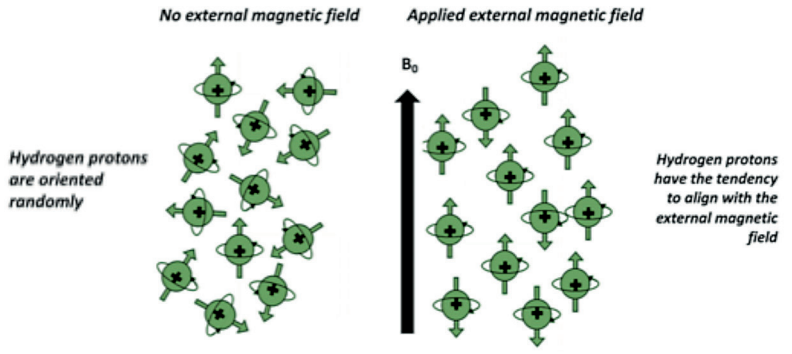


Figure 10 The hydrogen proton can be likened to the planet earth, spinning on its axis, with a north-south pole. In this respect it behaves like a small bar magnet. Under normal circumstances, these hydrogen proton "bar magnets" spin in the body with their axes randomly aligned. When the body is placed in a strong magnetic field, such as an MRI scanner, the protons' axes all line up. This uniform alignment creates a magnetic vector oriented along the axis of the MRI scanner.

Multiple transmitted RF-pulses can be used in sequence to emphasize particular tissues. A different emphasis occurs because different tissues (such as fat and water) have different relaxation times when the transmitted RF-pulse is switched off and can therefore be identified separately. The time taken for the protons to fully relax is measured in two ways. The first is the time taken for the magnetic vector to return to its resting state, called T1 relaxation, and the second is the time needed for the axial spin to return to its resting state, called T2 relaxation.

To produce a MR image, we also need to know what signal originates in which location. To this end, known variations are applied to the external magnetic field, in three orthogonal directions, making the signal place-dependent. This is achieved by the magnetic field gradients. The combination of spatial information from the gradient field and the signal intensity received after a series of RF-pulses allows a three-dimensional image to be reconstructed.

3.2.3. T1-weighted structural brain MRI

High-resolution three-dimensional T1-weighted MR images of the brain can be acquired to examine structural brain characteristics including GM volume, surface area, and cortical thickness (**fig. 11**). GM volume is the product of surface area and cortical thickness [153]. Cortical thickness and cortical surface area have a distinct genetic origin [153], and reflect different aspects of the underlying neural architecture [154]. Specifically, cortical thickness is believed to reflect the number of cells within the cortical columns, whereas cortical surface area is primarily determined by the number of columns within a cortical region [155]. GM volume reflects a combination of information obtained from several measures, as GM volume can reflect brain morphometric changes in both cortical and subcortical regions.

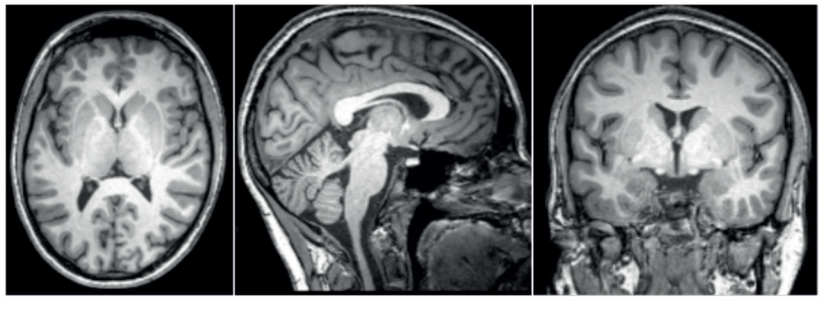


Figure 11 An example of high-resolution T1-weighted anatomical axial, sagittal, and coronal brain MR images acquired using a three-dimensional magnetization prepared rapid acquisition gradient echo (MP-RAGE), as acquired in this dissertation. Permission is obtained from the subject for presenting these images.

Therefore, it is valuable to combine GM volume and cortical thickness analyses together for a comprehensive evaluation of GM morphology. Voxel-based morphometry is frequently used to examine GM volume [156]. A voxel, is a volume element or a three-dimensional cube defined in a three-dimensional space such as the brain (three-dimensional equivalent of a pixel). Each voxel, depending on its size and location in the brain, contains thousands of neurons. In the present dissertation, all T1-weighted MR images were processed using FreeSurfer, a surface-based analysis method [157], which is based on vertices instead of voxels. A vertex is a point or intersection of six triangles, whereas a voxel is an intersection of grid lines. Surface models respect anatomical boundaries to a greater extent than voxelwise measures [158]. Noteworthy, various underlying molecular and cellular mechanisms may influence T1-weighted MRI signals (e.g. cell size, cell density, changes in dendritic spine structure, gliogenesis, synaptogenesis) [158].

3.2.4. Principles of Diffusion-Weighted MRI

DWI examines the diffusion of water molecules in the brain [159]. As a result of thermal energy, water molecules in the brain are in constant motion, which is called Brownian motion. DWI acquisition techniques make use of this Brownian motion to detect diffusion of water within and between individual cells. Diffusion can be appreciated when inserting a drop of ink in a glass of water; after some time, the ink molecules will gradually spread out. In an environment in which there are no obstacles in their path, such as in a glass of water, molecules jostling around due to thermal motion will disperse in a uniform manner, traveling an equal distance in all directions [160]. This is defined as *isotropic* diffusion. However, if the molecules encounter obstructions that are coherently oriented, they will no longer disperse equally in all directions, and diffusion will be *anisotropic*.

DW images can be analyzed using diffusion tensor analyzing techniques which model diffusion as a mathematical tensor [161]. The diffusion tensor can be represented as an ellipsoid whose main axis represents the principal direction of diffusion [162]. More specifically, the tensor is described by three orthogonal eigenvectors and their associated eigenvalues ($[lambda]_1$, $[lambda]_2$, $[lambda]_3$), and can be applied to each brain voxel [163], thereby characterizing the magnitude of water diffusion (mean diffusivity (MD)), its directional nonuniformity (fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD)), and its orientation (the tensor eigenvectors) [164]. In healthy WM, diffusion is more restricted perpendicular to an axon than parallel to it due to structural barriers [165].

The most commonly used DWI derived metric is FA [159], which ranges from 0 (maximal isotropic diffusion), meaning water molecules can diffuse equally in all directions (e.g. in cerebrospinal fluid), to 1 (maximal anisotropic diffusion), meaning the diffusion of water is hindered (e.g. movement is hindered perpendicular to the major axis of a WM tract) (**fig. 12 and 13**). FA is highly sensitive to microstructural WM changes, but not very specific to the type of changes [166].

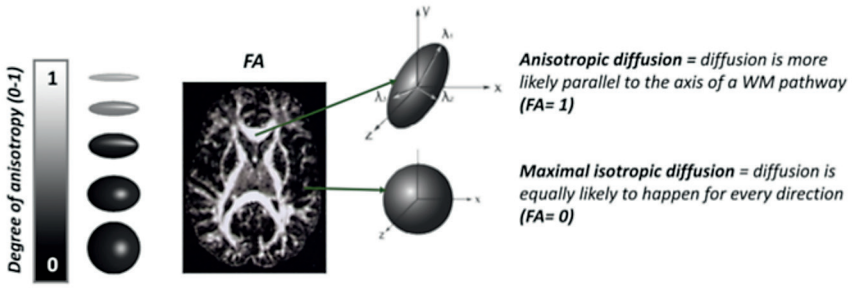


Figure 12 Axial fractional anisotropy map acquired with diffusion-weighted MRI, presenting anisotropic cylindrical diffusion (due to the underlying fiber architecture) in WM and isotropic spherical diffusion in GM.

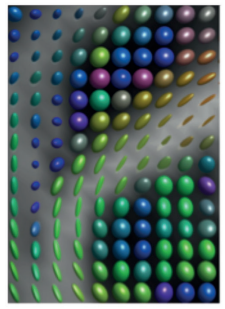


Figure 13 Representation of the directionality of water diffusion with cigar-shaped ellipsoids at the location of the WM pathway reflecting more diffusion parallel to the pathway and ball-shaped figures in GM to reflect multidirectional (isotropic) water diffusion. Figure from Alexander Leemans (http://www.exploredti.com).

3.2.5. Brain alterations in patients with chronic musculoskeletal pain

"Neuroplasticity contributes to both the constrained and unconstrained aspects of our nature. It renders our brains not only more resourceful, but also more vulnerable to outside influences."
- Norman Doidge

Research regarding brain alterations in patients with chronic INP and chronic WAD is essentially lacking, which is a prominent gap in current literature. To this end, a core aim of this dissertation was to investigate structural brain alterations in patients with chronic INP and chronic WAD. The current evidence with respect to structural and functional brain

alterations in chronic musculoskeletal pain patients including FM, and the relationship with clinical pain measures is systematically reviewed in part I of this thesis. Therefore, only a brief overview on this topic will be outlined in the following paragraph mainly focusing on the structural brain changes and providing a general overview of the somatosensory-discriminative, motivational-affective and cognitive aspects of pain.

During the past decades, a wide range of brain imaging techniques with a major role of MRI provided the opportunity to explore structural and functional alterations of the brain *in-vivo* in patients with chronic pain [167-169]. Subsequently, the role of maladaptive brain alterations, including GM volume alterations [85, 168, 169], changes in cortical thickness, and alterations in WM properties, have been gradually elucidated in the persistent pain and associated complaints of various chronic musculoskeletal pain conditions (e.g. FM [170-173], chronic low back pain [174-176], temporomandibular disorders [177, 178], chronic pelvic pain syndrome [179, 180]), and in mild traumatic brain injury (TBI) patients [181-183]. Especially, GM morphology and WM alterations in regions/tracts involved in cognitive processing and sensory-discriminative, affective and cognitive processing of pain have been shown in various chronic musculoskeletal pain syndromes, such as FM and chronic low back pain, characterized by the common pathophysiology of CS [170, 174].

Somatosensory-discriminative processing of pain refers to the possibility to discriminate different stimulus modalities such as pain quality, location, intensity, and duration. Brain regions such as the thalamus, the primary and secondary somatosensory cortex, the posterior insula, the posterior parietal cortex, and the mid-cingulate cortex are involved in this type of sensory-discriminative/ nociceptive pain processing [120, 184].

The *motivational-affective* (e.g. '*I am afraid that I will experience pain when I perform that cervical movement or activity*') *and cognitive dimensions* (e.g. '*When I experience pain this means that my neck is damaged*') of pain processing reflect the emotional experiences or value related to pain, as well as attention, cognitive appraisal of the meaning of the sensory signals, anticipation, and distraction influences of pain [185-187]. The anterior insula and the (dorsolateral, ventromedial) prefrontal cortex are for example associated with affective and cognitive processing of pain [188]. Pain often results in feelings of sadness, fear, and anger depending on how the pain is cognitively appraised [187]. Limbic brain regions are involved in various motivational-affective and cognitive components of pain processing such as the nucleus accumbens, amygdala, hippocampus, parahippocampal cortex, the (rostral) anterior and posterior cingulate cortex, and the orbitofrontal cortex [120, 169, 189].

The results of functional and structural brain MRI studies suggest that various chronic musculoskeletal pain conditions show a shift away from pain processing in somatosensory-discriminative/nociceptive brain regions towards crucial involvement of cognitive and motivational-affective processing of pain in regions often part of the limbic system and several regions involved in descending endogenous pain modulation [169, 188]. In addition,

chronic pain has a widespread impact on overall brain function, and disruptions in the activity of resting-state functional brain networks such as the default mode network may underlie the cognitive, affective-motivational and behavioral dysfunctions accompanying chronic musculoskeletal pain [190, 191].

Interestingly, previous brain imaging studies revealed associations between GM morphology (e.g. GM volume, cortical thickness) and WM microstructural changes, and measures of cognition and pain in various chronic pain conditions [170, 174, 177, 179, 192-194], and in mild TBI patients [183, 195]. Also, relationships between structural and functional brain alterations and features of cognitive emotional sensitization such as pain catastrophizing, and affective factors have been demonstrated in patients with chronic pain [196].

4. Cognition and chronic pain

Apart from persistent pain, chronic WAD and FM patients frequently complain of cognitive disturbances such as concentration and memory deficits [197-200]. Moreover, clinical experience emphasizes the possibility that chronic INP patients may also have some cognitive manifestations based on reporting of cognitive problems experienced during daily life. Nevertheless, in previous research, the nature and severity of self-perceived cognitive deficits and objective cognitive impairment has received less attention compared to pain mechanisms in chronic WAD, FM, and in particular in chronic INP. Cognitive function covers a broad range of mental processes performed by the brain, including attention, information processing, memory, learning, planning, organization, visuomotor tracking, decision making, cognitive flexibility, task-switching, cognitive inhibition, and executive functioning. Adequate cognitive performance is required to perform activities of daily living and more complex tasks such as during occupational activities. Accordingly, cognitive deficits can strongly interfere with daily life, social activities, and occupational performance [201]. FM patients rank cognitive dysfunction highly in terms of disease impact [202], and sometimes even report their forgetfulness, declines in memory and mental alertness, as more disturbing than persistent pain [203].

It has been reported that neural substrates of cognition and pain processing are linked, and that pain and cognition modulate one another reciprocally [201, 204]. Pain can negatively affect cognitive performance, and cognitively demanding tasks may reduce pain perception but possibly also may increase pain. The disruption of cognitive function by pain refers to the ability of pain to interfere and interrupt with one or more cognitive processes [201, 204].

Evidence exists for pain-related alterations in cognition, whereby anatomical, molecular and neurochemical features are common to both cognitive processing and supraspinal pain processing. Intriguingly, an overlap exists in brain regions involved in cognitive function and pain processing (e.g. amygdala, prefrontal cortex, hippocampus, anterior cingulate cortex) [204]. Pain-induced synaptic plasticity has also been shown to occur in brain regions with known roles in cognitive function such as the amygdala, anterior cingulate cortex, and hippocampus [106, 204]. Furthermore, several neurotransmitters are commonly involved in both pain processing and cognition [204]. For example, glutamate transmission through the NMDA receptor is essential for learning and memory through LTP [205]. Additionally, NMDA receptors are implicated in strengthened wind-up and CS [83].

Research has provided evidence for pain-related cognitive deficits in various chronic musculoskeletal pain conditions, including chronic WAD and FM [201]. Decreased cognitive function seems to be related to pain severity in patients with chronic WAD [199] and FM [206], and is presumed to be a feature of CS [60]. Although, there is increasing knowledge for the neurobiological overlap and relationship between cognitive function and CS, research exploring this relationship in patients with chronic musculoskeletal pain is essentially lacking.

Aims and Outline of the Dissertation

Aims and Outline of the Dissertation

The **core aim** of this dissertation was to improve our knowledge of the mechanisms that underlie the persistent, complex and often unexplained symptoms in patients with chronic INP, chronic WAD and FM. The core aim was furthermore to unravel differences in the nature and severity of underpinning mechanisms between these three chronic musculoskeletal pain conditions, displaying overlapping but also strikingly different features and clinical symptoms.

More specifically, the above outlined core aim is **threefold**:

- **Overall objective I:** to investigate the current evidence regarding associations between structural and functional brain alterations and clinical pain measures in chronic musculoskeletal pain patients, and regarding structural and functional brain alterations specifically in INP and WAD.
- **Overall objective II:** to examine differences between patients with idiopathic and traumatic chronic neck pain regarding disability, cognitive deficits, indices of CS, and structural brain alterations, and their interrelationships.
- **Overall objective III:** to explore the interaction between cognitive performance, cognitive stress, and CS in patients with chronic WAD and FM, thereby studying differences between these chronic pain disorders.

Part I focuses on **the first overall objective** and comprises *two chapters* including two systematic reviews. No clear overview was present on how brain alterations are related to clinical correlates of pain in various chronic musculoskeletal pain conditions. However, knowledge on this relationship is important to further disentangle the underpinning mechanisms of persistent pain.

Therefore, the ***aim*** of ***chapter 1*** was to perform a systematic review analyzing relationships between structural and functional brain alterations, and clinical pain measures in patients with chronic musculoskeletal pain, examined with structural and functional brain MRI techniques.

In addition, no review has summarized the evidence regarding brain alterations specifically in INP and WAD. Yet, a systematic review on this topic seemed warranted to identify the present state of the art and steer further research.

Accordingly, the ***aim*** of ***chapter 2*** was to systematically review the current evidence regarding structural and functional brain alterations in patients with acute, subacute, or chronic INP, and acute, subacute, or chronic WAD, examined with MRI techniques, Positron Emission Tomography, and Single-Photon Emission Computed Tomography. In addition, this study explored associations between brain alterations and clinical symptoms in these patients.

Part II includes three original research studies described in *three chapters* approaching **the second overall objective** of this dissertation. In particular, part II focuses on the examination of differences between patients with chronic INP and chronic WAD regarding

disability, cognitive deficits, CS, and structural brain alterations, and their interrelationships. In order to address this second overall objective, one large case-control study was performed, which only included adult women diagnosed as chronic INP or chronic WAD (WAD II A, B, or C according to the modified QTF scale) [28], and healthy pain-free women. In order to exclude the confounding factor of sex, only women were included, as research has demonstrated differences between men and women regarding brain structure, pain sensitivity and processing in healthy persons and pain patients. It can be hypothesized that the traumatic origin of neck pain in chronic WAD gives rise to more severe deficits compared to chronic INP. Nevertheless, studies exploring differences between both conditions remain scarce.

The *first* ***aim*** of ***chapter 3*** was to examine differences between patients with chronic INP and chronic WAD regarding disability, cognitive deficits, and CS encompassing hyperalgesia and CPM efficacy, compared to healthy controls. The *second aim* was to investigate relationships between measures of disability, cognitive deficits, and CS in both chronic neck pain conditions. The findings reported in *chapter 3* yield insight in one piece of the puzzle for understanding chronic INP and chronic WAD. However, another part of the puzzle for disentangling the underlying mechanisms could possibly be uncovered by performing brain MRI research in chronic INP and chronic WAD patients. Although it can be hypothesized that GM volume alterations are present in chronic WAD due to the traumatic event, because of cognitive deficits [198] and CS [46], but significantly less in chronic INP, this research is lacking.

To address the current research gap, the *first* ***aim*** of ***chapter 4*** was to examine GM volume alterations in brain regions involved in processing of cognition and pain in patients with chronic INP and chronic WAD compared to healthy controls. The *second aim* was to investigate associations between regional GM volume, and cognitive deficits, clinical pain measures, and indices of CS in both chronic neck pain conditions.

Furthermore, it could be hypothesized that alterations in cortical thickness and WM microstructure are associated with more severe cognitive deficits and CS in patients with chronic WAD compared to chronic INP. Nonetheless, these structural brain alterations, and the relationship with cognitive performance and CS, have never been investigated in these patients.

This brings us to ***chapter 5*** which ***aimed*** to investigate alterations in cortical thickness in regions involved in cognition or pain in patients with chronic WAD compared to chronic INP and controls. Secondly, this chapter *aimed* to examine abnormalities in WM microstructure, including alterations in FA, MD, AD, and RD in WM tracts carrying information between regions involved in cognition or pain in patients with chronic WAD compared to chronic INP and controls. In addition, the presence of brain microhemorrhages related to trauma was evaluated. Finally, the *purpose* of this chapter was to explore in each group separately whether alterations in regional cortical thickness and WM microstructure were

associated with cognitive performance and CS, encompassing distant hyperalgesia and CPM efficacy.

The third and last **overall objective** of this thesis is addressed in **part III**, which consists of *two chapters* enclosing a randomized crossover study exploring the interaction between cognitive performance, cognitive stress, and CS in patients with chronic WAD and FM. Besides the growing evidence for cognitive deficits, CS, and reduced quality of life in patients with chronic WAD and FM, studies examining the relationship between these features in both disorders are limited.

The *first* ***aim*** of ***chapter 6*** was to examine the presence of objective cognitive impairment using performance-based cognitive computer tests, signs of CS by experimentally assessing CPM and TS of second pain, and limitations on health-related quality of life in patients with chronic WAD and FM compared to controls. The *second aim* was to compare objective cognitive performance, signs of CS, and health-related quality of life between these two chronic pain conditions. The *final aim* was to investigate relationships between objective cognitive performance, and health-related quality of life and CS in patients with chronic WAD and FM, and controls. These objectives were tackled by performing the baseline case-control comparisons in the larger crossover study.

Furthermore, there is limited research concerning the influence of cognitive stress and relaxation on central pain modulation in patients with chronic WAD and FM. As such, it introduces the final manuscript of this thesis which is included in *chapter 7* and reports the results of the intervention part of the randomized crossover study.

The ***aim*** of ***chapter 7*** was to investigate the effect of performance-based cognitive tasks to induce cognitive stress, and a single progressive muscle relaxation session on central pain modulation encompassing TS of second pain and efficacy of CPM in patients with chronic WAD and FM compared to healthy individuals. Thereby, exploring interactions between cognitive stress and central pain modulation in both conditions characterized by CS.

In the general discussion, the most important findings are summarized and discussed. On the basis of these findings, clinical implications are formulated. Limitations and strengths of this dissertation are addressed and suggestions for further research are provided. The general discussion is ended with a general conclusion. Finally, a summary of the doctoral thesis both in English and Dutch is provided.

References General Introduction

References

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J* 2006; 15:834-848.
2. Murray CJ, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013; 369:448-457.
3. Hogg-Johnson S, van der Velde G, Carroll LJ, Holm LW, Cassidy JD, Guzman J, Cote P, Haldeman S, Ammendolia C, Carragee E, Hurwitz E, Nordin M, Peloso P. The burden and determinants of neck pain in the general population: results of the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated Disorders. *Spine (Phila Pa 1976)* 2008; 33:S39-51.
4. Carroll LJ, Hogg-Johnson S, van der Velde G, Haldeman S, Holm LW, Carragee EJ, Hurwitz EL, Cote P, Nordin M, Peloso PM, Guzman J, Cassidy JD. Course and prognostic factors for neck pain in the general population: results of the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated Disorders. *J Manipulative Physiol Ther* 2009; 32:S87-96.
5. Gore DR, Sepic SB, Gardner GM, Murray MP. Neck pain: a long-term follow-up of 205 patients. *Spine (Phila Pa 1976)* 1987; 12:1-5.
6. Hoy DG, Protani M, De R, Buchbinder R. The epidemiology of neck pain. *Best Pract Res Clin Rheumatol* 2010; 24:783-792.
7. Hoy D, March L, Woolf A, Blyth F, Brooks P, Smith E, Vos T, Barendregt J, Blore J, Murray C, Burstein R, Buchbinder R. The global burden of neck pain: estimates from the global burden of disease 2010 study. *Ann Rheum Dis* 2014; 73:1309-1315.
8. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388:1545-1602.
9. Verhagen AP, Scholten-Peeters GG, van Wijngaarden S, de Bie RA, Bierma-Zeinstra SM. Conservative treatments for whiplash. *Cochrane Database Syst Rev* 2007:Cd003338.
10. Manchikanti L, Singh V, Datta S, Cohen SP, Hirsch JA. Comprehensive review of epidemiology, scope, and impact of spinal pain. *Pain Physician* 2009; 12:E35-70.
11. Borghouts JA, Koes BW, Bouter LM. The clinical course and prognostic factors of non-specific neck pain: a systematic review. *Pain* 1998; 77:1-13.
12. Barnsley L, Lord S, Bogduk N. Whiplash injury. *Pain* 1994; 58:283-307.
13. van Randeraad-van der Zee CH, Beurskens AJ, Swinkels RA, Pool JJ, Batterham RW, Osborne RH, de Vet HC. The burden of neck pain: its meaning for persons with neck pain and healthcare providers, explored by concept mapping. *Qual Life Res* 2016; 25:1219-1225.
14. Jull G SM, Falla D, Treleaven J, O'Leary S. Sensory manifestations of neck pain, in Elsevier CL (ed). Whiplash, headache, and neck pain: Research-based directions for physical therapies. Sarena Wolfaard, 2008, pp 5.
15. Jull G SM, Falla D, Treleaven J, O'Leary S. Whiplash, headache, and neck pain. Research-based directions for physical therapies. Sarena Wolfaard, Churchill Livingstone Elsevier, 2008.
16. De Pauw R, Coppieters I, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? - A systematic review. *Man Ther* 2015.
17. Gwendolen J SM, Falla D, Treleaven J, O'Leary S. Whiplash, headache and neck pain. Sarena Wolfaard, 2008.
18. Dewitte V, Peersman W, Danneels L, Bouche K, Roets A, Cagnie B. Subjective and clinical assessment criteria suggestive for five clinical patterns discernible in nonspecific neck pain patients. A Delphi-survey of clinical experts. *Man Ther* 2016; 26:87-96.
19. Castelein B, Cools A, Parlevliet T, Cagnie B. Are chronic neck pain, scapular dyskinesis and altered scapulothoracic muscle activity interrelated?: A case-control study with surface and fine-wire EMG. *J Electromyogr Kinesiol* 2016; 31:136-143.
20. Stanton TR, Leake HB, Chalmers KJ, Moseley GL. Evidence of Impaired Proprioception in Chronic, Idiopathic Neck Pain: Systematic Review and Meta-Analysis. *Phys Ther* 2016; 96:876-887.
21. Falla D, Bilenkij G, Jull G. Patients with chronic neck pain demonstrate altered patterns of muscle activation during performance of a functional upper limb task. *Spine (Phila Pa 1976)* 2004; 29:1436-1440.
22. Chien A, Sterling M. Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. *Man Ther* 2010; 15:48-53.
23. Dimitriadis Z, Kapreli E, Strimpakos N, Oldham J. Do psychological states associate with pain and disability in chronic neck pain patients? *J Back Musculoskelet Rehabil* 2015; 28:797-802.
24. Malfliet A, Kregel J, Cagnie B, Kuipers M, Dolphens M, Roussel N, Meeus M, Danneels L, Bramer WM, Nijs J. Lack of evidence for central sensitization in idiopathic, non-traumatic neck pain: a systematic review. *Pain Physician* 2015; 18:223-236.
25. Davis CG. Mechanisms of chronic pain from whiplash injury. *J Forensic Leg Med* 2013; 20:74-85.
26. Elliott JM, Noteboom JT, Flynn TW, Sterling M. Characterization of acute and chronic whiplash-associated disorders. *J Orthop Sports Phys Ther* 2009; 39:312-323.
27. Seroussi R, Singh V, Fry A. Chronic whiplash pain. *Phys Med Rehabil Clin N Am* 2015; 26:359-373.
28. Sterling M. A proposed new classification system for whiplash associated disorders--implications for assessment and management. *Man Ther* 2004; 9:60-70.
29. Rodriquez AA, Barr KP, Burns SP. Whiplash: pathophysiology, diagnosis, treatment, and prognosis. *Muscle Nerve* 2004; 29:768-781.
30. Sterling M, Jull G, Vicenzino B, Kenardy J, Darnell R. Physical and psychological factors predict outcome following whiplash injury. *Pain* 2005; 114:141-148.
31. Hendriks EJ, Scholten-Peeters GG, van der Windt DA, Neeleman-van der Steen CW, Oostendorp RA, Verhagen AP. Prognostic factors for poor recovery in acute whiplash patients. *Pain* 2005; 114:408-416.
32. Rebbeck T, Sindhusake D, Cameron ID, Rubin G, Feyer AM, Walsh J, Gold M, Schofield WN. A prospective cohort study of health outcomes following whiplash associated disorders in an Australian population. *Inj Prev* 2006; 12:93-98.
33. Carroll LJ, Holm LW, Hogg-Johnson S, Cote P, Cassidy JD, Haldeman S, Nordin M, Hurwitz EL, Carragee EJ, van der Velde G, Peloso PM, Guzman J. Course and prognostic factors for neck pain in whiplash-associated disorders (WAD): results of the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated Disorders. *Spine (Phila Pa 1976)* 2008; 33:S83-92.
34. Kamper SJ, Rebbeck TJ, Maher CG, McAuley JH, Sterling M. Course and prognostic factors of whiplash: a systematic review and meta-analysis. *Pain* 2008; 138:617-629.
35. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician* 2015; 18:E389-401.
36. Sterner Y, Gerdle B. Acute and chronic whiplash disorders--a review. *J Rehabil Med* 2004; 36:193-209; quiz 210.
37. Wenzel HG, Haug TT, Mykletun A, Dahl AA. A population study of anxiety and depression among persons who report whiplash traumas. *J Psychosom Res* 2002; 53:831-835.
38. Sterling M. Whiplash-associated disorder: musculoskeletal pain and related clinical findings. *J Man Manip Ther* 2011; 19:194-200.
39. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine (Phila Pa 1976)* 1995; 20:1s-73s.
40. Peolsson M, Borsbo B, Gerdle B. Generalized pain is associated with more negative consequences than local or regional pain: a study of chronic whiplash-associated disorders. *J Rehabil Med* 2007; 39:260-268.
41. Valenza MC, Valenza G, Gonzalez-Jimenez E, De-la-Llave-Rincon AI, Arroyo-Morales M, Fernandez-de-Las-Penas C. Alteration in sleep quality in patients with mechanical insidious neck pain and whiplash-associated neck pain. *Am J Phys Med Rehabil* 2012; 91:584-591.
42. Sterling M. A proposed new classification system for whiplash associated disorders—implications for assessment and management. *Man Ther* 2004; 9:60-70.
43. Sterling M, Jull G, Vicenzino B, Kenardy J, Darnell R. Development of motor system dysfunction following whiplash injury. *Pain* 2003; 103:65-73.
44. Sterling M, Jull G, Vicenzino B, Kenardy J. Sensory hypersensitivity occurs soon after whiplash injury and is associated with poor recovery. *Pain* 2003; 104:509-517.
45. Sterling M, Kenardy J, Jull G, Vicenzino B. The development of psychological changes following whiplash injury. *Pain* 2003; 106:481-489.
46. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013; 17:299-312.

47. Sterling M, Jull G, Vicenzino B, Kenardy J. Sensory hypersensitivity occurs soon after whiplash injury and is associated with poor recovery. *Pain* 2003; 104:509-517.
48. Stone AM, Vicenzino B, Lim EC, Sterling M. Measures of central hyperexcitability in chronic whiplash associated disorder--a systematic review and meta-analysis. *Man Ther* 2013; 18:111-117.
49. Jones GT, Atzeni F, Beasley M, Fluss E, Sarzi-Puttini P, Macfarlane GJ. The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol* 2015; 67:568-575.
50. Marques AP, Santo AS, Berssaneti AA, Matsutani LA, Yuan SL. Prevalence of fibromyalgia: literature review update. *Rev Bras Reumatol* 2016.
51. Mease P. Fibromyalgia syndrome: review of clinical presentation, pathogenesis, outcome measures, and treatment. *J Rheumatol Suppl* 2005; 75:6-21.
52. Mease P, Arnold LM, Choy EH, Clauw DJ, Crofford LJ, Glass JM, Martin SA, Morea J, Simon L, Strand CV, Williams DA. Fibromyalgia syndrome module at OMERACT 9: domain construct. *J Rheumatol* 2009; 36:2318-2329.
53. Clauw DJ. Fibromyalgia: a clinical review. *Jama* 2014; 311:1547-1555.
54. Staud R. Chronic widespread pain and fibromyalgia: two sides of the same coin? *Curr Rheumatol Rep* 2009; 11:433-436.
55. Clauw DJ. Fibromyalgia and related conditions. *Mayo Clin Proc* 2015; 90:680-692.
56. Sluka KA, Clauw DJ. Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience* 2016; 338:114-129.
57. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 1990; 33:160-172.
58. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res (Hoboken)* 2010; 62:600-610.
59. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Hauser W, Katz RL, Mease PJ, Russell AS, Russell IJ, Walitt B. 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum* 2016; 46:319-329.
60. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Man Ther* 2010; 15:135-141.
61. Nijs J, Torres-Cueco R, van Wilgen CP, Girbes EL, Struyf F, Roussel N, van Oosterwijck J, Daenen L, Kuppens K, Vanwerweeen L, Hermans L, Beckwee D, Voogt L, Clark J, Moloney N, Meeus M. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. *Pain Physician* 2014; 17:447-457.
62. Merskey H Bogduk N. Classification of Chronic Pain. Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms. , in Press I (ed). International Association for the Study of Pain (ed 2nd ed.), Seatle, 1994.
63. Smart KM, Blake C, Staines A, Doody C. Clinical indicators of 'nociceptive', 'peripheral neuropathic' and 'central' mechanisms of musculoskeletal pain. A Delphi survey of expert clinicians. *Man Ther* 2010; 15:80-87.
64. Bouhassira D. [Definition and classification of neuropathic pain]. *Presse Med* 2008; 37:311-314.
65. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 2011; 152:S2-15.
66. Nijs J, Malfliet A, Ickmans K, Baert I, Meeus M. Treatment of central sensitization in patients with 'unexplained' chronic pain: an update. *Expert Opin Pharmacother* 2014; 15:1671-1683.
67. Woolf CJ. What to call the amplification of nociceptive signals in the central nervous system that contribute to widespread pain? *Pain* 2014; 155:1911-1912.
68. Staud R, Smitherman ML. Peripheral and central sensitization in fibromyalgia: pathogenetic role. *Curr Pain Headache Rep* 2002; 6:259-266.
69. Staud R, Robinson ME, Price DD. Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. *J Pain* 2007; 8:893-901.
70. Nielsen LA, Henriksson KG. Pathophysiological mechanisms in chronic musculoskeletal pain (fibromyalgia): the role of central and peripheral sensitization and pain disinhibition. *Best Pract Res Clin Rheumatol* 2007; 21:465-480.
71. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. . *Pain Supplement* 1986; 3:S1-226.
72. Williams AC, Craig KD. Updating the definition of pain. *Pain* 2016; 157:2420-2423.
73. Purves D AG, Fitzpatrick D, Katz LC, LaMantia A- S. McNamara. . Pain. , in Purves D AG, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara (ed). Neuroscience (ed 3th). Sunderland: Sinauer Associations, 1997., pp 167.
74. Winkelstein BA. Mechanisms of central sensitization, neuroimmunology & injury biomechanics in persistent pain: implications for musculoskeletal disorders. *J Electromyogr Kinesiol* 2004; 14:87-93.
75. Staud R. Evidence of involvement of central neural mechanisms in generating fibromyalgia pain. *Curr Rheumatol Rep* 2002; 4:299-305.
76. Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001; 413:203-210.
77. Perl ER, Kumazawa T, Lynn B, Kenins P. Sensitization of high threshold receptors with unmyelinated (C) afferent fibers. *Prog Brain Res* 1976; 43:263-277.
78. Bishop T, Marchand F, Young AR, Lewin GR, McMahon SB. Ultraviolet-B-induced mechanical hyperalgesia: A role for peripheral sensitisation. *Pain* 2010; 150:141-152.
79. Fernandez-de-Las-Penas C, Simons D, Cuadrado ML, Pareja J. The role of myofascial trigger points in musculoskeletal pain syndromes of the head and neck. *Curr Pain Headache Rep* 2007; 11:365-372.
80. Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. *Pain* 1979; 6:249.
81. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 2006; 10:287-333.
82. Harstall C OM. How prevalent is chronic pain? *Pain Clinical Updates* 2003; 11:1-4.
83. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain* 2009; 10:895-926.
84. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000; 288:1765-1769.
85. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. *Journal of Neuroimmune Pharmacology* 2013; 8:518-534.
86. Affaitati G, Costantini R, Fabrizio A, Lapenna D, Tafuri E, Giamberardino MA. Effects of treatment of peripheral pain generators in fibromyalgia patients. *Eur J Pain* 2011; 15:61-69.
87. Seybold VS. The role of peptides in central sensitization. *Handb Exp Pharmacol* 2009:451-491.
88. Chiang CY, Li Z, Dostrovsky JO, Sessle BJ. Central sensitization in medullary dorsal horn involves gap junctions and hemichannels. *Neuroreport* 2010; 21:233-237.
89. Rivera-Arconada I, Lopez-Garcia JA. Changes in membrane excitability and potassium currents in sensitized dorsal horn neurons of mice pups. *J Neurosci* 2010; 30:5376-5383.
90. Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, Curatolo M. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. *Pain* 2004; 107:7-15.
91. Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. *Prog Neurobiol* 1992; 38:397-421.
92. Arendt-Nielsen L. Central sensitization in humans: assessment and pharmacology. *Handb Exp Pharmacol* 2015; 227:79-102.
93. AI. B. Memories of pain. *Science Medicine* 1996; 3:22-31.
94. Koltzenburg M, Torebjork HE, Wahren LK. Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. *Brain* 1994; 117 (Pt 3):579-591.
95. Sandkuhler J. Models and mechanisms of hyperalgesia and allodynia. *Physiol Rev* 2009; 89:707-758.
96. Yarnitsky D. Role of endogenous pain modulation in chronic pain mechanisms and treatment. *Pain* 2015; 156 Suppl 1:S24-31.
97. Mendell LM, Wall PD. RESPONSES OF SINGLE DORSAL CORD CELLS TO PERIPHERAL CUTANEOUS UNMYELINATED FIBRES. *Nature* 1965; 206:97-99.
98. Price DD, Hu JW, Dubner R, Gracely RH. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. *Pain* 1977; 3:57-68.
99. Mendell LM. Physiological properties of unmyelinated fiber projection to the spinal cord. *Exp Neurol* 1966; 16:316-332.
100. Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurones and pain sensation: much ado about something? *Prog Neurobiol* 2000; 61:169-203.
101. Staud R, Craggs JG, Perlstein WM, Robinson ME, Price DD. Brain activity associated with slow temporal summation of C-fiber evoked pain in fibromyalgia patients and healthy controls. *Eur J Pain* 2008; 12:1078-1089.

102. Staud R. Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. *Expert Rev Neurother* 2012; 12:577-585.
103. Staud R, Craggs JG, Robinson ME, Perlstein WM, Price DD. Brain activity related to temporal summation of C-fiber evoked pain. *Pain* 2007; 129:130-142.
104. Liu XG, Zhou LJ. Long-term potentiation at spinal C-fiber synapses: a target for pathological pain. *Curr Pharm Des* 2015; 21:895-905.
105. Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* 2003; 26:696-705.
106. Zhuo M. A synaptic model for pain: long-term potentiation in the anterior cingulate cortex. *Mol Cells* 2007; 23:259-271.
107. Bennett GJ. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA-receptor. *J Pain Symptom Manage* 2000; 19:S2-6.
108. Meller ST, Gebhart GF. Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain* 1993; 52:127-136.
109. Luo ZD, Cizkova D. The role of nitric oxide in nociception. *Curr Rev Pain* 2000; 4:459-466.
110. Liu H, Brown JL, Jasmin L, Maggio JE, Vigna SR, Mantyh PW, Basbaum AI. Synaptic relationship between substance P and the substance P receptor: light and electron microscopic characterization of the mismatch between neuropeptides and their receptors. *Proc Natl Acad Sci U S A* 1994; 91:1009-1013.
111. Bruel-Jungerman E, Davis S, Laroche S. Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 2007; 13:492-505.
112. Zusman M. Forebrain-mediated sensitization of central pain pathways: 'non-specific' pain and a new image for MT. *Man Ther* 2002; 7:80-88.
113. Nijs J, Lluch Girbes E, Lundberg M, Malfliet A, Sterling M. Exercise therapy for chronic musculoskeletal pain: Innovation by altering pain memories. *Man Ther* 2015; 20:216-220.
114. Vaziri S, Serak J, Wang M. Long-term potentiation of pain "memory" in the spinal cord. *Neurosurgery* 2015; 76:N14-15.
115. Kucyi A, Davis KD. The dynamic pain connectome. *Trends Neurosci* 2015; 38:86-95.
116. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. *Brain Res Rev* 2009; 60:214-225.
117. Curatolo M, Arendt-Nielsen L, Petersen-Felix S. Central hypersensitivity in chronic pain: mechanisms and clinical implications. *Phys Med Rehabil Clin N Am* 2006; 17:287-302.
118. Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; 66:355-474.
119. Brosschot JF. Cognitive-emotional sensitization and somatic health complaints. *Scand J Psychol* 2002; 43:113-121.
120. Bushnell MC, Ceko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. *Nat Rev Neurosci* 2013; 14:502-511.
121. Rygh LJ, Tjolsen A, Hole K, Svendsen F. Cellular memory in spinal nociceptive circuitry. *Scand J Psychol* 2002; 43:153-159.
122. Le Bars D, Villanueva L. Electrophysiological evidence for the activation of descending inhibitory controls by nociceptive afferent pathways. *Prog Brain Res* 1988; 77:275-299.
123. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, Hansson P, Lautenbacher S, Marchand S, Wilder-Smith O. Recommendations on terminology and practice of psychophysical DNIC testing. *Eur J Pain* 2010; 14:339.
124. Moont R, Pud D, Sprecher E, Sharvit G, Yarnitsky D. 'Pain inhibits pain' mechanisms: Is pain modulation simply due to distraction? *Pain* 2010; 150:113-120.
125. Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D. Temporal changes in cortical activation during conditioned pain modulation (CPM), a LORETA study. *Pain* 2011; 152:1469-1477.
126. Potvin S, Marchand S. Pain facilitation and pain inhibition during conditioned pain modulation in fibromyalgia and in healthy controls. *Pain* 2016; 157:1704-1710.
127. Daenen L, Nijs J, Roussel N, Wouters K, Van Loo M, Cras P. Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clin Rheumatol* 2013; 32:23-31.
128. Kosek E, Hansson P. Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. *Pain* 1997; 70:41-51.

129. Curatolo M, Arendt-Nielsen L, Petersen-Felix S. Evidence, mechanisms, and clinical implications of central hypersensitivity in chronic pain after whiplash injury. *Clin J Pain* 2004; 20:469-476.
130. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP. Central hypersensitivity in chronic pain after whiplash injury. *Clin J Pain* 2001; 17:306-315.
131. Chua NHL, Timmerman H, Vissers KC, Wilder-Smith OH. Multi-modal Quantitative Sensory Testing in Patients with Unilateral Chronic Neck Pain: An Exploratory Study. *Journal of Musculoskeletal Pain* 2012; 20:292-299.
132. Butler RK, Finn DP. Stress-induced analgesia. *Prog Neurobiol* 2009; 88:184-202.
133. Olango WM, Finn DP. Neurobiology of stress-induced hyperalgesia. *Curr Top Behav Neurosci* 2014; 20:251-280.
134. Buijs RM, Van Eden CG. The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system. *Prog Brain Res* 2000; 126:117-132.
135. Woda A, Picard P, Dutheil F. Dysfunctional stress responses in chronic pain. *Psychoneuroendocrinology* 2016; 71:127-135.
136. McLean SA, Clauw DJ, Abelson JL, Liberzon I. The development of persistent pain and psychological morbidity after motor vehicle collision: integrating the potential role of stress response systems into a biopsychosocial model. *Psychosom Med* 2005; 67:783-790.
137. Suarez-Roca H, Leal L, Silva JA, Pinerua-Shuhaibar L, Quintero L. Reduced GABA neurotransmission underlies hyperalgesia induced by repeated forced swimming stress. *Behav Brain Res* 2008; 189:159-169.
138. Quintero L, Cardenas R, Suarez-Roca H. Stress-induced hyperalgesia is associated with a reduced and delayed GABA inhibitory control that enhances post-synaptic NMDA receptor activation in the spinal cord. *Pain* 2011; 152:1909-1922.
139. Gaab J, Baumann S, Budnoik A, Gmunder H, Hottinger N, Ehlert U. Reduced reactivity and enhanced negative feedback sensitivity of the hypothalamus-pituitary-adrenal axis in chronic whiplash-associated disorder. *Pain* 2005; 119:219-224.
140. Wingenfeld K, Heim C, Schmidt I, Wagner D, Meinlschmidt G, Hellhammer DH. HPA axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. *Psychosom Med* 2008; 70:65-72.
141. Neeck G, Crofford LJ. Neuroendocrine perturbations in fibromyalgia and chronic fatigue syndrome. *Rheum Dis Clin North Am* 2000; 26:989-1002.
142. Martenson ME, Cetas JS, Heinricher MM. A possible neural basis for stress-induced hyperalgesia. *Pain* 2009; 142:236-244.
143. Khasar SG, Burkham J, Dina OA, Brown AS, Bogen O, Alessandri-Haber N, Green PG, Reichling DB, Levine JD. Stress induces a switch of intracellular signaling in sensory neurons in a model of generalized pain. *J Neurosci* 2008; 28:5721-5730.
144. Barakat A, Vogelzangs N, Licht CM, Geenen R, MacFarlane GJ, de Geus EJ, Smit JH, Penninx BW, Dekker J. Dysregulation of the autonomic nervous system and its association with the presence and intensity of chronic widespread pain. *Arthritis Care Res (Hoboken)* 2012; 64:1209-1216.
145. Tak LM, Riese H, de Bock GH, Manoharan A, Kok IC, Rosmalen JG. As good as it gets? A meta-analysis and systematic review of methodological quality of heart rate variability studies in functional somatic disorders. *Biol Psychol* 2009; 82:101-110.
146. Crofford LJ. The hypothalamic-pituitary-adrenal stress axis in fibromyalgia and chronic fatigue syndrome. *Z Rheumatol* 1998; 57 Suppl 2:67-71.
147. Riva R, Mork PJ, Westgaard RH, Ro M, Lundberg U. Fibromyalgia syndrome is associated with hypocortisolism. *Int J Behav Med* 2010; 17:223-233.
148. Pedler A, Sterling M. Patients with chronic whiplash can be subgrouped on the basis of symptoms of sensory hypersensitivity and posttraumatic stress. *Pain* 2013; 154:1640-1648.
149. Sterling M. Does knowledge of predictors of recovery and nonrecovery assist outcomes after whiplash injury? *Spine (Phila Pa 1976)* 2011; 36:S257-262.
150. Mansour AR, Farmer MA, Baliki MN, Apkarian AV. Chronic pain: the role of learning and brain plasticity. *Restor Neurol Neurosci* 2014; 32:129-139.
151. L. NW. Introduction to Brain Anatomy, in Miller K (ed). Biomechanics of the brain. Springer, 2011, pp 5-40.
152. Berger A. Magnetic resonance imaging. *Bmj* 2002; 324:35.

153. Winkler AM, Kochunov P, Blangero J, Almasy L, Zilles K, Fox PT, Duggirala R, Glahn DC. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *Neuroimage* 2010; 53:1135-1146.
154. Rakic P. Defects of neuronal migration and the pathogenesis of cortical malformations. *Prog Brain Res* 1988; 73:15-37.
155. Rakic P. Evolution of the neocortex: a perspective from developmental biology. *Nat Rev Neurosci* 2009; 10:724-735.
156. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000; 11:805-821.
157. Fischl B. FreeSurfer. *Neuroimage* 2012; 62:774-781.
158. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat Neurosci* 2012; 15:528-536.
159. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J* 1994; 66:259-267.
160. Louise Emsell WVH, Jacques-Donald Tournier. Introduction to Diffusion Tensor Imaging, in Wim van Hecke LE, Stefan Sunaert (ed). Chapter 2: Diffusion Tensor Imaging: A practical handbook. Springer, New york Heidelberg Dordrecht London, 2016, pp 7-19.
161. Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. *J Magn Reson B* 1994; 103:247-254.
162. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B* 1996; 111:209-219.
163. Johansen-Berg H, Rushworth MF. Using diffusion imaging to study human connectional anatomy. *Annu Rev Neurosci* 2009; 32:75-94.
164. K M. Curran LE, A. Leemans. Quantitative DTI measures, in Wim van Hecke LE, Stefan Sunaert (ed). Diffusion tensor imaging: a practical handbook. Springer, New York, 2016, pp 65-85.
165. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed* 2002; 15:435-455.
166. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. *Neurotherapeutics* 2007; 4:316-329.
167. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain* 2011; 152:S49-64.
168. Schmidt-Wilcke T. Neuroimaging of chronic pain. *Best Pract Res Clin Rheumatol* 2015; 29:29-41.
169. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 2015; 87:474-491.
170. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Semin Arthritis Rheum* 2014; 44:68-75.
171. Kim DJ, Lim M, Kim JS, Son KM, Kim HA, Chung CK. Altered white matter integrity in the corpus callosum in fibromyalgia patients identified by tract-based spatial statistical analysis. *Arthritis Rheumatol* 2014; 66:3190-3199.
172. Lieberman G, Shpaner M, Watts R, Andrews T, Filippi CG, Davis M, Naylor MR. White Matter Involvement in Chronic Musculoskeletal Pain. *Journal of Pain* 2014; 15:1110-1119.
173. Lutz J, Jager L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis Rheum* 2008; 58:3960-3969.
174. Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B. Structural and functional brain abnormalities in chronic low back pain: A systematic review. *Semin Arthritis Rheum* 2015.
175. Mansour AR, Baliki MN, Huang L, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Brain white matter structural properties predict transition to chronic pain. *Pain* 2013; 154:2160-2168.
176. Buckalew N, Haut MW, Aizenstein H, Morrow L, Perera S, Kuwabara H, Weiner DK. Differences in brain structure and function in older adults with self-reported disabling and nondisabling chronic low back pain. *Pain Med* 2010; 11:1183-1197.
177. Younger JW, Shen YF, Goddard G, Mackey SC. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. *Pain* 2010; 149:222-228.
178. Moayedi M, Weissman-Fogel I, Salomons TV, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. White matter brain and trigeminal nerve abnormalities in temporomandibular disorder. *Pain* 2012; 153:1467-1477.

179. Mordasini L, Weisstanner C, Rummel C, Thalmann GN, Verma RK, Wiest R, Kessler TM. Chronic pelvic pain syndrome in men is associated with reduction of relative gray matter volume in the anterior cingulate cortex compared to healthy controls. *J Urol* 2012; 188:2233-2237.
180. Huang L, Kutch JJ, Ellingson BM, Martucci KT, Harris RE, Clauw DJ, Mackey S, Mayer EA, Schaeffer AJ, Apkarian AV, Farmer MA. Brain white matter changes associated with urological chronic pelvic pain syndrome: multisite neuroimaging from a MAPP case-control study. *Pain* 2016; 157:2782-2791.
181. Govindarajan KA, Narayana PA, Hasan KM, Wilde EA, Levin HS, Hunter JV, Miller ER, Patel VK, Robertson CS, McCarthy JJ. Cortical Thickness in Mild Traumatic Brain Injury. *J Neurotrauma* 2016.
182. Wang X, Xie H, Cotton AS, Tamburrino MB, Brickman KR, Lewis TJ, McLean SA, Liberzon I. Early cortical thickness change after mild traumatic brain injury following motor vehicle collision. *J Neurotrauma* 2015; 32:455-463.
183. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. *Brain* 2007; 130:2508-2519.
184. Wager TD, Atlas LY, Lindquist MA, Roy M, Woo CW, Kross E. An fMRI-based neurologic signature of physical pain. *N Engl J Med* 2013; 368:1388-1397.
185. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron* 2008; 60:570-581.
186. Hashmi JA, Baliki MN, Huang L, Baria AT, Torbey S, Hermann KM, Schnitzer TJ, Apkarian AV. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain* 2013; 136:2751-2768.
187. Garland EL. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. *Prim Care* 2012; 39:561-571.
188. Tracey I. Getting the pain you expect: mechanisms of placebo, nocebo and reappraisal effects in humans. *Nat Med* 2010; 16:1277-1283.
189. Vachon-Presseau E, Centeno MV, Ren W, Berger SE, Tetreault P, Ghantous M, Baria A, Farmer M, Baliki MN, Schnitzer TJ, Apkarian AV. The Emotional Brain as a Predictor and Amplifier of Chronic Pain. *J Dent Res* 2016; 95:605-612.
190. Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci* 2008; 28:1398-1403.
191. Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional reorganization of the default mode network across chronic pain conditions. *PLoS One* 2014; 9:e106133.
192. Coppieters I, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. *J Pain* 2016.
193. Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain* 2008; 131:3222-3231.
194. Moayedi M, Weissman-Fogel I, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. *Neuroimage* 2011; 55:277-286.
195. List J, Ott S, Bukowski M, Lindenberg R, Floel A. Cognitive function and brain structure after recurrent mild traumatic brain injuries in young-to-middle-aged adults. *Front Hum Neurosci* 2015; 9:228.
196. Malfliet A, Coppieters I, Van Wilgen P, Kregel J, De Pauw R, Dolphens M, Ickmans K. Brain changes associated with cognitive and emotional factors in chronic pain: A systematic review. *Eur J Pain* 2017.
197. Seo J, Kim SH, Kim YT, Song HJ, Lee JJ, Kim SH, Han SW, Nam EJ, Kim SK, Lee HJ, Lee SJ, Chang Y. Working memory impairment in fibromyalgia patients associated with altered frontoparietal memory network. *PLoS One* 2012; 7:e37808.
198. Kessels RP, Aleman A, Verhagen WI, van Luijtelaar EL. Cognitive functioning after whiplash injury: a meta-analysis. *J Int Neuropsychol Soc* 2000; 6:271-278.
199. Antepohl W, Kiviloog L, Andersson J, Gerdle B. Cognitive impairment in patients with chronic whiplash-associated disorder--a matched control study. *NeuroRehabilitation* 2003; 18:307-315.
200. McAllister SJ, Toussaint LL, Williams DA, Hoskin TL, Whipple MO, Vincent A. Perceived dyscognition reported by patients with fibromyalgia. *Clin Exp Rheumatol* 2016; 34:S48-54.
201. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. *Prog Neurobiol* 2011; 93:385-404.

202. Kravitz HM, Katz RS. Fibrofog and fibromyalgia: a narrative review and implications for clinical practice. *Rheumatol Int* 2015; 35:1115-1125.
203. Arnold LM, Crofford LJ, Mease PJ, Burgess SM, Palmer SC, Abetz L, Martin SA. Patient perspectives on the impact of fibromyalgia. *Patient Educ Couns* 2008; 73:114-120.
204. Moriarty O, Finn DP. Cognition and pain. *Current Opinion in Supportive and Palliative Care* 2014; 8:130-136.
205. Rison RA, Stanton PK. Long-term potentiation and N-methyl-D-aspartate receptors: foundations of memory and neurologic disease? *Neurosci Biobehav Rev* 1995; 19:533-552.
206. Reyes Del Paso GA, Pulgar A, Duschek S, Garrido S. Cognitive impairment in fibromyalgia syndrome: the impact of cardiovascular regulation, pain, emotional disorders and medication. *Eur J Pain* 2012; 16:421-429.

References General Introduction

PART I

Is chronic musculoskeletal pain associated with structural and functional brain alterations?

Systematic literature reviews

Chapter 1

Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: a systematic review

Coppieters I. MSc[1, 2], Meeus M. PhD[1, 2, 3], Kregel J. MSc[1, 2, 4], Caeyenberghs K. PhD[5], De Pauw R. MSc[1], Goubert D. MSc[1, 4], Cagnie B. PhD[1]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium
[2] Pain in Motion international research group, www.paininmotion.be
[3] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium
[4] Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Faculty of Physical Education & Physiotherapy, Free University Brussels, Belgium
[5] School of Psychology, Faculty of Health Sciences, Australian Catholic University, Australia

The Journal of Pain, 2016; 17: 949-962.
SCI_{2015}= 4.463, 5-year impact factor= 4.759, Q1 in clinical neurology (31/193), Q1 in neurosciences (56/256)

Abstract

Background and aims: Compelling evidence has shown chronic widespread and exaggerated pain experience in chronic musculoskeletal pain (MSKP) conditions. In addition, neuroimaging research has revealed morphological and functional brain alterations in these patients. It is hypothesized that brain alterations play a role in the persistent pain complaints of patients with chronic MSKP. Nevertheless, lack of overview exists regarding the relations between brain alterations and clinical measures of pain. The present systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines, to investigate the relations between structural or functional brain alterations, using magnetic resonance imaging scans, and clinical pain measures in patients with chronic MSKP.

Methods: PubMed, Web of Science, Cinahl, and Cochrane databases were searched. First, the obtained articles were screened according to title and abstract. Second, the screening was on the basis of full-text. Risk of bias in included studies was investigated according to the modified Newcastle-Ottawa Scale.

Results: Twenty studies met the inclusion criteria. Moderate evidence shows that higher pain intensity and pressure pain sensitivity are related to decreased regional gray matter (GM) volume in brain regions encompassing the cingulate cortex, the insula, and the superior frontal and temporal gyrus. Further, some evidence exists that longer disease duration in fibromyalgia is correlated with decreased total GM volume. Yet, inconclusive evidence exists regarding the association of longer disease duration with decreased or increased regional GM volume in other chronic MSKP conditions. Inconclusive evidence was found regarding the direction of the relation of pain intensity and pressure pain sensitivity with microstructural white matter and functional connectivity alterations.

Conclusion: In conclusion, preliminary to moderate evidence demonstrates relations between clinical pain measures, and structural and functional connectivity alterations within brain regions involved in somatosensory, affective, and cognitive processing of pain in chronic MSKP. Nevertheless, inconclusive results exist regarding the direction of these relations. Further research is warranted to unravel whether these brain alterations are positively or negatively correlated to clinical pain measures.

Perspective: *Structural and functional brain alterations within regions involved in somatosensory, affective, and cognitive pain processing play a crucial role in the persistent pain of chronic MSKP patients. Accordingly, these brain alterations have to be taken into account when assessing and treating patients with chronic MSKP.*

Key words: Chronic musculoskeletal pain, magnetic resonance imaging, brain alterations, pain intensity, pain duration.

Introduction

Chronic musculoskeletal pain (MSKP) is defined as pain in muscles, tendons, joints, and ligaments for >3 months.[24] Additionally, this condition is frequently characterized by disproportional pain, meaning that pain severity and dysfunction are disproportionate to the nature and extent of the musculoskeletal damage/deficit.[77] Accordingly, increasing evidence suggests that most of these chronic MSKP syndromes are related to disturbed central pain processes and not strict to peripheral structures.[75] Chronic MSKP conditions include temporomandibular disorders, idiopathic chronic low back pain, fibromyalgia, chronic pelvic pain, and chronic whiplash-associated disorders, among others.[14,17]

Chronic MSKP affects hundreds of millions of people worldwide and is one of the most common forms of chronic pain.[95] This chronic pain condition can cause a profound negative effect on an individual's physical, emotional, and social well-being and thus on quality of life.[90,95] Additionally, MSKP syndromes result in a major burden on health systems, and social care systems, resulting in substantial financial costs.[95]

Accumulating research has shown that features of central sensitization are often present in these chronic MSKP conditions.[20,25,29,81,92] Central sensitization can be defined as an augmented responsiveness of the central nervous system to nociceptive as well as nonnociceptive stimuli (eg, pain, electrical stimuli, pressure, and temperature).[76,80] This exaggerated responsiveness can cause allodynia, hyperalgesia, hypersensitivity of senses unrelated to the musculoskeletal system, and referred pain across multiple spinal segments, leading to chronic widespread pain.[66] Clinical measures/symptoms of central sensitization are for example widespread pain, heightened pain intensity, generalized hyperalgesia, and allodynia.[77]

Approximately 30 years ago noninvasive human brain imaging techniques emerged.[2] This advent provided the opportunity to examine brain structure and function in clinical chronic pain states. During the past decade, the role of the brain in chronic pain conditions has been gradually elucidated.[82] This neuroimaging research has shown neuronal plasticity in the brain, which can lead to maladaptive changes due to sustained abnormal nociceptive input.[3,26] Specifically, the brain of patients suffering from chronic pain displays alterations with respect to brain structure,[5,56] function,[44] and chemistry.[33] In addition, neuroplastic brain remodeling can lead to persistence of pain, even in the absence of (further) nociceptive input.[3,11] Emerging evidence suggests that chronic pain is associated with a distinct representation in the brain, which is often referred to as the neural pain signature.[54]

Magnetic resonance imaging (MRI) has been one of the most influential techniques that has led to an improved understanding of pain perception, modulation, and chronification.[26,82] Brain MRI techniques can be roughly divided into structural and functional MRI (fMRI).

Structural MRI has the ability to measure gray matter (GM) and white matter (WM) morphology in vivo. High-resolution T1-weighted images can be used to assess global measures, such as whole brain volume, GM volume,[30] as well as regional features, including surface area,[31] cortical thickness,[30] and regional GM volume.[6] Voxel-based morphometry is frequently used to examine GM volume and is a voxel-wise comparison of the local concentration of GM between different groups.[6] FreeSurfer is used to render construction of cortical surface models, volumetric segmentation of brain structures, and mapping of cortical GM thickness (http://freesurfer.net, v5.3.0).[30] Three-dimensional mapping of cortical thickness is also possible using the Laplace equation method.[47]

Diffusion MRI is an innovative technique to investigate WM properties and micro-structural WM changes.[7] Diffusion imaging data are used to map the 3-dimensional diffusion of water molecules in the brain. Currently, diffusion tensor imaging (DTI) is the most widely used method for assessing WM orientation and integrity. The diffusion tensor characterizes the degree, the magnitude of anisotropy, and the orientation of directional diffusion.[1] This technique provides measures such as fractional anisotropy (FA) and mean-, radial-, and axial diffusivity (AD).[87] Nevertheless, it recently became clear that this tensor model is invalid in voxels containing crossing fibers.[89] Therefore, various methods have been developed that are capable to extract multiple fiber orientations from the diffusion-weighted imaging signal, thereby overcoming the limitation of DTI.[45] Compelling structural MRI research has shown alterations in GM morphology and WM properties in various chronic MSKP conditions, including patients with fibromyalgia, chronic low back pain, and chronic temporomandibular disorders,[20,52,61,68] within brain regions involved in somatosensory, affective, and cognitive modulation of pain, such as the somatosensory cortex (S1, S2), medial and dorsolateral prefrontal cortex, anterior (ACC) and posterior cingulate cortex (PCC), insula, amygdala, hippocampus, and periaqueductal gray.[8,19-21,27,37,52,82,83]

Functional MRI (fMRI) is used to evaluate human brain function in vivo and is on the basis of measuring the blood oxygen-level dependent (BOLD) contrast.[15] fMRI is able to analyze changes in BOLD contrast during a task or during rest.[13] During task-based fMRI, the BOLD contrast shows the hemodynamic brain changes after enhanced neural brain activity while performing a specific task.[59,78] When a person is at rest (no task), spontaneous low-frequency (< .1 Hz) fluctuations of the BOLD signal occur throughout the brain.[38] This signal exposes temporal correlations in spatially distinct brain regions. Certain patterns appear consistently and are referred to as resting-state functional networks.

Resting-state fMRI research aims to generate statistical maps of these significant temporal BOLD correlations between brain areas.[13] These correlations of signal fluctuations between distinct brain regions are calculated as an index of functional connectivity (FC).[34] The default mode network (DMN) and the salience network are examples of resting-state (no task) functional networks in the brain.[54] Recently, various research groups have shown reorganized connectivity patterns within various resting-state functional networks such as the DMN and salience network in chronic pain patients.[9,40,55,64] Resting-state fMRI and FC analyses have improved our knowledge on how brain regions work together as networks to modulate pain and how these networks may be modified in the presence of persistent pain.[18,82]

fMRI research has shown alterations in (resting-state) functional activity and connectivity within various brain regions involved in somatosensory, affective, and cognitive modulation of pain in patients with various chronic MSKP conditions.[9,10,20,52]

During the past years, the relationship between brain alterations and clinical features of pain has been frequently hypothesized and studied.[26] Scientific evidence for underlying central mechanisms of pain processing has become increasingly necessary. Imaging studies investigating the relation between brain alterations and clinical behavioral measures in chronic MSKP patients have been published during the past years.[82] In particular, accumulating research investigating the relationship between clinical measures of pain such as pain duration, pain intensity, pressure pain sensitivity, and brain alterations has been published.[37,48,60] Evaluation of clinical pain measures is highly important and is often used in clinical assessment, therapy, and research in patients with chronic MSKP to evaluate the nature and extent of symptoms as well as the evolution of pain.[36,57] A systematic review has shown that clinical pain outcome measures, used in research to assess chronic MSKP, are very heterogeneous.[57] In particular, various dimensions of pain, different types of scales/questionnaires and descriptors, and varying reporting periods of pain (eg, current pain intensity, mean pain intensity during past week or past month) are examined in chronic MSKP research. Overall, clinical pain measures can be subdivided into self-reported pain measures such as pain intensity and pain duration, and more objective experimental pain measures such as pressure pain sensitivity and hypersensitivity for various stimuli.[57]

Unfortunately, currently no clear overview exists on how brain alterations are related to clinical correlates of pain in various chronic MSKP conditions. However, knowledge on this relationship is important to integrate neuroimaging findings into clinical practice and to further unravel the underlying mechanisms of persistent pain. Therefore, the aim of the present systematic review was to investigate the relations between structural and functional brain alterations and clinical pain measures in chronic MSKP patients, examined with structural and functional brain MRI techniques.

Methods

Research Questions

This systematic review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines.[70] The Patient, measurement Instrument, Comparison, Outcome (PICO) approach was applied to formulate the following research

questions: 1) 'What are the relations between structural brain alterations (O = outcome) and clinical pain measures (O) in chronic MSKP patients (P = patient), examined with structural brain MRI techniques (I = measurement instrument)? 2) 'What are the relations between functional brain alterations (O) and clinical pain measures (O) in chronic MSKP patients (P), examined with functional brain MRI techniques (I)?

Eligibility Criteria

Eligibility assessment was performed by screening the obtained articles on the basis of the inclusion and exclusion criteria (Table 1). To be included, articles had to investigate a relation or association between structural or functional brain alterations and clinical measures/correlates of pain (ie, pain duration, pain intensity, pain perception, pressure sensitivity, hyperalgesia, hypersensitivity, allodynia, referred pain) (O) by using brain MRI techniques (I) in patients with chronic MSKP (P).

Table 1 Inclusion and Exclusion criteria

	Inclusion	*Exclusion*
Population	· Human study · Patients with chronic MSKP · Adults (≥ 18 y of age)	· Animal study · Children and adolescents (<18 y of age)
Instrument	· At least 1 brain MRI technique is applied: T1 MRI, DTI, DWI, fMRI, rs-fMRI	· SPECT, PET, EEG, MEG, MR spectroscopy
Outcome 1	· At least 1 clinical pain measure was examined: pain intensity, pain perception, pain duration, allodynia, hyperalgesia, referred pain, pressure sensitivity	· Not examining the relation, association, or correlation between a clinical pain measure and structural or functional brain alterations
Outcome 2	· At least 1 type of brain change was examined: structural or functional alterations	· Not examining the relation, association, correlation between a clinical pain measure and brain alterations
Type of report	· Clinical · Full-text	· Nonclinical: review, systematic review, meta-analysis, letters to the editor · Full-text not available, abstracts, posters
Language	· English, German, Dutch, French	· All other languages

Abbreviations: T1 MRI, T1-weighted MRI; DWI, diffusion weighted imaging; rs-fMRI, resting-state fMRI; SPECT, single photon emission computed tomography; PET, photon emission tomography; EEG, electroencephalography; MEG, magnetoencephalography; MR, magnetic resonance.

Eligibility assessment of the obtained articles was performed by 2 independent researchers (I.C. and B.C.), who have published systematic reviews and were trained in conducting a systematic review by the second author (M.M.). After deduplication, a first screening was performed on the basis of the title and abstract of the remaining articles. If any of the inclusion criteria were not met, the article was excluded. In the second phase, publications were screened on the basis of the full-text and fulfilment of the inclusion criteria was ensured.

Literature Search Strategy

A systematic search of relevant literature was conducted by the authors. The electronic databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Web of Science (http://isiwebofknowledge.com), Cinahl (https://health.ebsco.com/products/cinahl-complete), and Cochrane (http://onlinelibrary.wiley.com/cochranelibrary/search) were searched through on September 7, 2015 to identify relevant articles. To make the search as complete as possible, reference lists of the included articles were screened. The search strategy consisted of a combination of free text words on the basis of the eligibility criteria. The complete search strategy is shown in Table 2.

Risk of Bias in Individual Studies

Methodological quality of all included studies was assessed by 2 independent reviewers (I.C. and J.K.), both PhD candidates working with chronic MSKP patients in the research field of brain MRI. Both reviewers were trained by M.M., a PhD experienced in conducting systematic reviews. Risk of bias was evaluated using the Newcastle-Ottawa Scale (NOS) for case-control studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).[93] The NOS applies a star rating system to judge methodological quality on the basis of 3 subcategories: selection of groups, comparability, and ascertainment of exposure. This checklist is recommended for case-control studies[99] and has frequently been used by the Cochrane Collaboration (www.cochrane.org). The criterion on response rate could not be scored because this item was not applicable for the articles on the current research topic. Therefore, item 9 was replaced by a self-constructed additional subcategory, 'MRI data quality and preprocessing' that includes 2 items, which was chosen specifically for the current systematic review. Item 9 scores whether the researchers performed visual inspection of the MRI data quality (eg, head motion). Item 10 scores whether manual exclusion in case of low data quality and/or data adjustment was included in the preprocessing pipeline. Subsequently, each study could reach a maximum score of 10 on the modified NOS, representing the highest methodological quality. A study earned 1 point when controlling for sex or age in the 'comparability' section and an additional point when controlling for another factor (eg, medication use, collecting cardiorespiratory data).

Table 2 The search terms (free text words) used for the literature review

Patients	*AND*	***Measurement Instrument***	*AND*	***Outcome***	*AND*	***Outcome***
chronic musculoskeletal pain syndrome		brain imaging		white matter		allodynia
chronic low back pain		diffusion tensor imaging		cortical thickness		hyperalgesia
fibromyalgia		diffusion weighted imaging		grey matter		heightened sensitivity
chronic fatigue syndrome		magnetization transfer contrast		gray matter		hypersensitivity
temporomandibular disorders		magnetization transfer ratio		grey matter volume		referred pain
osteoarthritis		T1		gray matter volume		pain duration
chronic knee pain		voxel-based morphometry		functional connectivity		pain severity
chronic pelvic pain syndrome		functional magnetic resonance imaging		structural connectivity		pain intensity
chronic ankle pain		fMRI		resting state activity		
chronic neck pain		resting-state functional magnetic resonance imaging		resting state connectivity		
chronic whiplash associated disorder		resting state fMRI		cortical morphology		
chronic epicondylalgia		tractography				
Myofacial pain syndrome						

On the basis of study design and methodological quality, each individual study received a level of evidence, according to the 2005 classification system of the Dutch Institute for Healthcare Improvement (CBO) (Supplementary Table 1). Subsequently, strength of conclusion was determined after clustering studies with comparable experimental methods and research aims, accounting for the study design and the risk of bias (Supplementary Table 2). Strength of conclusion 2 was assigned when there were at least 2 independently conducted studies of evidence level B. Strength of conclusion 3 was assigned when there was at least 1 study of evidence level B. Strength of conclusion 4 was given in case of inconclusive or inconsistent results between various studies.

Data Extraction Process

The following information was extracted from each included study and is shown in the evidence table (Supplementary Table 3): 1) patients, 2) control group, 3) brain MRI technique, 4) clinical pain measures, 5) correlations, relations, associations, 6) main results, and 7) correlation coefficients, t-scores, Z-scores. The data were obtained by the first author (I.C.) and a second reviewer (R.D.P.) checked the extracted data. Noteworthy, the evidence table only includes the MRI techniques and clinical pain measures, which were used to evaluate possible relations. In addition, the main results regarding relations between clinical pain measures and brain alterations in chronic MSKP patients are summarized whereas the results among the healthy control group are not shown.

Results

Study Selection

The selection process of relevant articles is presented in Figure 1. The initial search resulted in 137 articles. After removing the duplicates, 91 articles remained. Two articles[32,37] were found by manual search: these articles were found in the reference list of included studies. The entire selection process resulted in 20 eligible articles.

Study Characteristics

All included studies (n = 20) applied a case-control design, comparing chronic MSKP patients with healthy pain-free individuals. The characteristics of each study were extracted and presented in the evidence table (Supplementary Table 3). Articles were divided on the basis of the applied MRI technique. Six articles compared clinical pain measures with GM alterations,[22,37,53,67,71,97] 4 articles with WM alterations,[48,56,61,68] and 11 articles observed relations with functional brain alterations.[9,22,28,32,43,44,49,51,60,74,98]

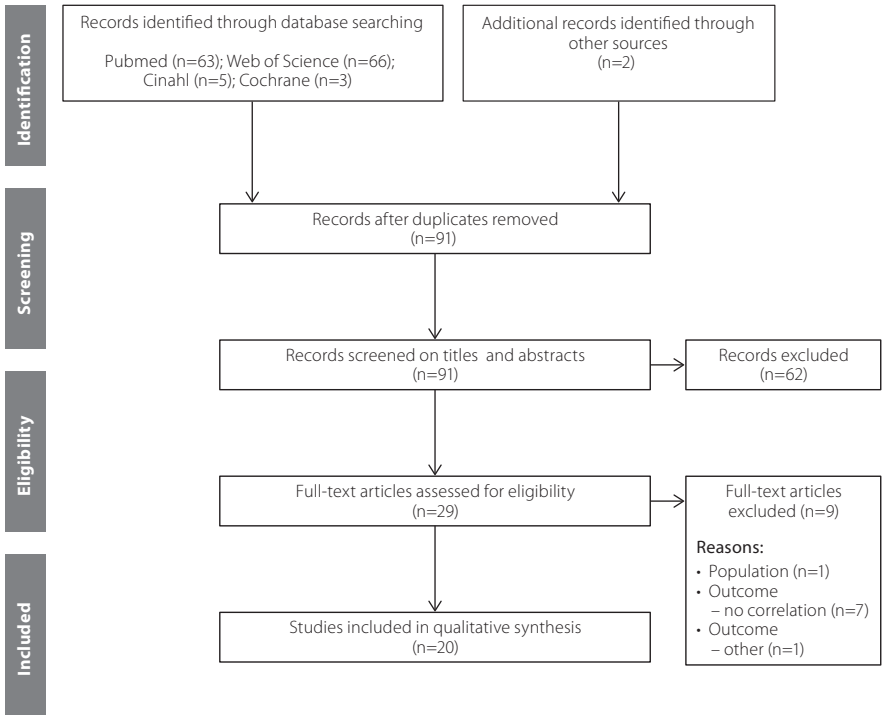


Figure 1 Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram of the conducted search.

Risk of Bias Within Studies and Level of Evidence

The risk of bias and level of evidence is shown in Table 3. All studies scored a level of evidence B. Methodological quality was moderate to good, varying between 5 of 10 (50%) and 9 of 10 (90%). Most studies lost points on 'representativeness of the cases' (80%), 'selection of controls' (85%), and 'definition of controls' (60%), either because authors did not mention the required information or the information was not adequate. Nevertheless, most studies were awarded for taking into account confounding factors (eg, matching for age and sex), ascertainment of exposure, and for using the same method of ascertainment for cases and controls. All studies were awarded for manual exclusion in case of low data quality and/or inclusion of automated data adjustment in the preprocessing pipeline.

In most cases (90.5% or 181 of the 200 items), the 2 reviewers (I.C. and J.K.) agreed. After a second review and a comparison of the 19 differences, the reviewers reached a consensus for 197 items. For the 3 remaining items, a third investigator was consulted (R.D.P.). The final score of each study is presented in Table 3.

Table 3 Methodological Quality for Case-Control Studies

Study	Selection				Comparability		Exposure		MRI data quality and pre-processing		Total score (%)	LOE
	1	2	3	4	5	6	7	8	9	10		
Kim et al[48]	-	+	+	+	+	+	+	+	+	+	**9/10** (90)	B
Moayedi et al[68]	+	/	-	+	+	+	+	+	+	+	**8/10** (80)	B
Ichesco et al[44]	+	-	-	+	+	+	+	+	+	+	**8/10** (80)	B
Ichesco et al[43]	+	-	-	+	+	+	+	+	+	+	**8/10** (80)	B
Gerstner et al[37]	+	-	-	+	+	+	+	+	-	+	**8/10** (80)	B
Lutz et al[61]	+	/	-	+	+	+	+	+	+	+	**8/10** (80)	B
Moayedi et al[67]	+	-	-	+	+	+	+	+	-	+	**7/10** (70)	B
Kim et al[49]	+	-	-	+	+	+	+	+	-	+	**7/10** (70)	B
López-Solà et al[60]	+	/	-	-	+	+	+	+	+	+	**7/10** (70)	B
Baliki et al[10]	+	+	-	-	+	+	+	+	-	+	**7/10** (70)	B
Mordasini et al[71]	+	-	-	-	+	+	+	+	+	+	**7/10** (70)	B
Yu et al[98]	+	-	+	-	+	+	+	-	-	+	**7/10** (60)	B
Ceko et al[22]	+	-	-	-	+	+	+	+	-	+	**7/10** (70)	B
Kong et al[51]	+	-	+	-	+	+	+	-	+	+	**7/10** (70)	B
Flodin et al[32]	-	-	-	-	+	+	+	+	+	+	**6/10** (60)	B
Farmer et al[28]	+	+	-	-	+	+	+	+	-	+	**6/10** (60)	B
Lieberman et al[56]	+	-	-	-	+	-	+	+	+	+	**6/10** (60)	B
Younger et al[96]	+	+	-	-	+	-	/	+	-	+	**5/10** (50)	B
Napadow et al[74]	-	-	-	-	+	+	+	+	-	+	**5/10** (50)	B
Kuchinad et al[53]	-	-	-	-	+	+	+	+	-	+	**5/10** (50)	B

Abbreviations: LOE, level of evidence; +, score fulfilled; -, score not fulfilled; /, answer is unclear.
NOTE. Newcastle-Ottawa Quality Assessment Scale: case-control studies: 1=Is the case definition adequate?; 2=Representativeness of the cases; 3=Selection of controls; 4=Definition of controls; 5=Study controls for age or sex; 6=study controls for any additional factor; 7=Ascertainment of exposure; 8=Same method of ascertainment for cases and controls; 9= visual inspection of the MRI data quality; 10= manual exclusion in case of low data quality and/or automated data adjustment included in pre-processing pipeline.

Syntheses of Results

Structural Brain MRI

Overall, 10 studies investigated the relationship between structural brain alterations and clinical pain measures in chronic MSKP patients.[22,37,48,53,56,61,67,68,71,97] Six of 10 articles used voxel-based morphometry,[22,37,53,67,71,97] 1 article performed cortical thickness analysis,[67] and 4 articles applied DTI.[48,56,61,68]

GM Alterations Related to Clinical Pain Measures

Pain Intensity. Three studies examined the relation between clinical pain intensity and alterations in regional GM volume.[67,71,97]

Mordasini et al[71] and Younger et al[96] reported a significant relation between pain intensity and regional GM volume. Increased pain intensity in patients with chronic temporomandibular disorders was associated with decreased GM volume in the right rostral ACC, right PCC, precuneus, and superior frontal and superior temporal gyrus.[97] Mordasini et al[71] reported correlations between higher chronic pelvic pain intensity and decreased GM volume in the left ACC.

In conclusion, there is moderate evidence that higher clinical pain intensity in chronic MSKP patients is related to decreased GM volume in pain processing regions such as the ACC[71,97] (strength of conclusion 2).

Moayedi et al[67] reported a negative correlation between pain intensity in temporomandibular disorders patients and GM thickness in the anterior midcingulate cortex and the ventrolateral aspect of the primary motor cortex. Furthermore, they reported that increased pain unpleasantness was associated with decreased GM thickness in the lateral orbitofrontal cortex.

In conclusion, there is some evidence that increased pain intensity and pain unpleasantness in chronic temporomandibular disorders patients is correlated with decreased GM thickness in pain, motor, and cognitive processing regions of the brain (strength of conclusion 3).

Pressure Pain Sensitivity

Two studies reported an association between pressure pain sensitivity and regional GM volume alterations.[22,97]

Younger et al[96] reported a negative association between pressure pain sensitivity and GM volume in the trigeminal nuclei in chronic temporomandibular disorders patients. Furthermore, Ceko and colleagues[22] observed significant relations between increased pressure pain sensitivity and decreased GM volume in the left anterior insula and PCC in fibromyalgia patients.

In conclusion, there is moderate evidence that increased pressure pain sensitivity in chronic MSKP patients is associated with decreased GM volume in somatosensory, pain, and affect-cognitive processing brain regions (strength of conclusion 2).

Pain Duration

Four articles[37,53,67,97] reported an association between the duration of chronic MSKP and GM volume alterations. Three articles described a relation with regional GM volume[37,67,97] and 1 article showed a relation with total GM volume.[53] A negative correlation was reported by Gerstner et al,[37] who observed that longer pain duration in chronic temporomandibular disorders patients was correlated with decreased GM volume in the right superior and middle temporal gyrus. Kuchinad et al[53] reported that longer disease duration in fibromyalgia patients was correlated with decreased total GM volume. In contrast, Younger et al[96] and Moayedi et al[67] described a positive relation between duration of temporomandibular disorders and regional GM volume. Increased GM volume was found in the PCC and midbrain bilaterally, in the right hippocampus and in the right middle cerebellar peduncle.[97] Further, longer temporomandibular disorders disease duration was correlated with increased GM volume in the sensory thalamus.[67]

In conclusion, there is moderate evidence that regional GM volume alterations are correlated with chronic MSKP duration (strength of conclusion 2). However, inconclusive evidence exists regarding the relation between longer disease duration and decreased or increased regional GM volume (strength of conclusion 4). Additionally, there is some evidence that longer disease duration in fibromyalgia patients is correlated with decreased total GM volume (strength of conclusion 3).

WM Alterations Related to Clinical Pain Measures

Pain Intensity. Four studies[48,56,61,68] investigated the relationship between clinical pain correlates and structural alterations in WM using DTI as an MRI technique. Kim and colleagues[48] and Moayedi and colleagues[68] reported a correlation between higher pain intensity and lower FA in the corpus callosum,[48] internal, external and extreme capsules,[68] and the thalamus.[68] Moayedi et al[68] also detected a negative correlation between pain unpleasantness and FA in the right internal capsule. In contrast, Lutz et al[61] and Lieberman et al[56] have reported positive correlations between higher pain intensity and increased FA values in the WM of the right superior frontal gyrus[61] and between higher total pain experience score and increased FA in the left uncinate fasciculus.[56]

In conclusion, there is moderate evidence that higher pain intensity is correlated with FA alterations in regional WM tracts involved in transmission of somatosensory, pain, and affective and cognitive information (strength of conclusion 2). However, there is inconclusive evidence as to whether greater pain intensity is related to decreased or increased FA values in these WM tracts (strength of conclusion 4).

Subjective Pain Scores

Lieberman et al[56] reported positive correlations in chronic MSKP patients between higher total pain experience scores and increased AD in the left anterior and posterior limb of the internal capsule. Additionally, increased typical pain scores on the McGill pain questionnaire were positively correlated with increased AD in the left anterior limb.

In conclusion, there is some evidence that increased subjective pain scores in chronic MSKP patients are correlated with increased AD in WM tracts involved in transmission of information through the anterior and posterior limb of the internal capsule (strength of conclusion 3).

Functional Brain MRI

Overall, 11 articles described interrelations between clinical pain correlates and functional brain alterations using fMRI and/or resting-state fMRI in chronic MSKP patients.[9,22,28,32,43,44,49,51,60,74,98] Six studies examined fibromyalgia patients, 1 article included chronic pelvic pain patients, 3 articles assessed chronic low back pain patients, 1 article investigated osteoarthritis patients, and 1 article included patients with temporomandibular disorders.

FC Alterations Related to Clinical Pain Measures

Pain Intensity. Most studies investigated relations between clinical pain measures and FC alterations.[9,22,32,43,44,49,51,73,74,98] Napadow et al[74] reported in fibromyalgia patients a positive association between higher current pain intensity and increased FC between the DMN and right middle and anterior insula, cerebellum, dorsolateral prefrontal cortex, and subgenual ACC. Further, a positive covariation was reported between higher current pain intensity and increased FC between the right executive attention network and right anterior, left middle, and posterior insula and putamen.

In contrast, Napadow et al[74] reported higher current pain intensity to be related to decreased FC between the right executive attention network and the hippocampus, periaqueductal gray, nucleus cuneiformis, and the pontine raphe. A negative relation was shown in temporomandibular disorders patients between pain intensity and FC between the left anterior insula and rostral ACC during resting-state fMRI by Ichesco et al.[43] Ichesco et al,[44] reported in fibromyalgia patients higher pain intensity to be related to increased FC between the right anterior insula and superior temporal gyrus. Kong et al[51] reported positive relations between pain intensity changes after exercises and FC at the left insula, precuneus, amygdala, and fusiform in chronic low back pain patients.

Baliki et al[10] reported in chronic low back pain and osteoarthritis patients positive correlations between current pain intensity and medial prefrontal cortex/insula FC. Further, Ceko et al[22] reported in fibromyalgia patients positive relations between current pain intensity and FC of the left anterior insula to the primary somatosensory cortex (S1) and primary motor cortex. In addition, Kim et al[49] reported a correlation between higher pain intensity and increased changes (from the pain phase through the rest phase) in S1 leg connectivity to the anterior insula in fibromyalgia patients. Additionally, increased temporal summation of pain was correlated with increased changes in S1 leg connectivity to the right anterior/middle insula in fibromyalgia patients. In contrast, higher clinical pain intensity was related to decreased resting-state FC within S1.

Ichesco et al[44] examined associations between pain rating index scores and FC in fibromyalgia patients. Higher FC, between insula and superior temporal gyrus, was associated with higher affective scores. Higher sensory scores were correlated with greater FC between the right middle insula and bilateral precuneus. In contrast, Yu et al[98] observed in chronic low back pain patients a negative relationship between increased low back pain ratings and FC between periaqueductal gray and left ventromedial prefrontal cortex/rostral ACC after a pain-inducing maneuver.

In conclusion, there is moderate evidence that greater clinical pain intensity is related to alterations in FC in chronic MSKP patients (strength of conclusion 2). However, inconclusive evidence exists regarding the direction of the relation within somatosensory, pain, and affect-cognitive processing regions/networks in chronic MSKP patients. Positive[9,22,32,44,51,73,74] and negative[32,43,49,73,74,98] relations between pain intensity and FC alterations were found (strength of conclusion 4).

Pressure Pain Sensitivity

Three studies investigated the relation between pressure pain sensitivity and FC alterations.[32,43,44] Flodin et al[32] reported that increased pressure pain sensitivity in fibromyalgia was correlated with decreased FC between the right inferior orbitofrontal regions and right associative visual cortex. In contrast, they also reported a relation between increased pressure pain sensitivity and increased FC between pain-related regions (ie, the left insula and dorsal PCC, the left Rolandic operculum, left parahippocampal gyrus, and thalamus and prefrontal cortex).

Ichesco et al[44] investigated correlations between FC and pressure pain thresholds at different intensities. In fibromyalgia patients, a negative correlation was detected between lower pressure pain thresholds, hence increased pressure pain sensitivity and higher FC. Higher FC was reported between the right posterior insula and PCC during a faint, mild, and slightly intense stimulus. A slightly intense stimulus correlated with FC between the left middle insula and left middle cingulate cortex. When a faint stimulus was given, higher FC was reported between the left middle insula and right middle cingulate cortex and between the right posterior insula and left middle ACC. Ichesco et al[43] reported that increased pressure pain sensitivity was related to decreased FC between the left anterior insula and the right ACC and medial frontal gyrus in chronic temporomandibular disorders patients.

In conclusion, there is moderate evidence that pressure pain sensitivity is related to alterations in FC within somatosensory, pain, and affect-cognitive processing brain regions/networks in chronic MSKP patients (strength of conclusion 2). However, inconclusive evidence exists regarding the direction of the relation between increased pressure pain sensitivity and FC alterations in chronic MSKP patients. Positive[32,43] and negative[32,44] relations between pressure pain sensitivity and FC alterations were found (strength of conclusion 4).

Functional Activity Alterations Related to Clinical Pain Measures

Pain Intensity. Farmer et al[28] reported a positive correlation between pain intensity and activity in the anterior insula in men with chronic pelvic pain. Lopez-Sola et al[60] observed negative correlations between pain intensity in fibromyalgia patients and activation in primary and secondary visual cortical areas. Furthermore, hypersensitivity to tactile

stimulation (ie, allodynia) was related to decreased activation in the superior middle temporal gyri.

In conclusion, there is some evidence that higher pain intensity and allodynia are associated with decreased functional brain activation in fibromyalgia patients (strength of conclusion 3). Further, there is some evidence that greater pain intensity is related to increased functional activity in the anterior insula in men with chronic pelvic pain (strength of conclusion 3).

Discussion

The purpose of this systematic review was to summarize the evidence regarding relations between structural and functional brain alterations and clinical pain measures in chronic MSKP patients, examined with brain MRI techniques. Most studies reported significant relations between structural and functional alterations in the brain and various clinical pain correlates such as pain intensity, pain duration, and pressure pain sensitivity. Overall, the included studies examined a wide range of brain regions involved in somatosensory, cognitive, and affective processing of pain. Remarkably, the direction of the relations (eg, increased or decreased GM volume related to higher pain measures) often differed between and within various studies. This might be due to a variety of conditions that are classified as chronic MSKP, together with the multiple MRI acquisition and analytical techniques that have been applied to measure alterations in the brain. Furthermore, the different standardized scales and questionnaires that have been used to measure clinical features of pain could have influenced the direction and nature of the observed relations as well as the specific brain regions that were investigated. Nevertheless, several conclusions can be made and are summarized in Table 4. In addition, a glossary of important terms regarding MRI analysis of brain alterations is presented in Table 5.

Twenty case-control studies met the inclusion criteria. All studies scored a level of evidence B. Methodological quality was moderate to good, varying between 5 of 10 (50%) and 9 of 10 (90%). Moderate evidence shows that higher pain intensity and pressure pain sensitivity are related to decreased regional GM volume in brain regions encompassing the cingulate cortex, the insula, and the superior frontal and temporal gyrus.[22,71,97] Further, some evidence exists that longer disease duration in fibromyalgia patients is correlated with decreased total GM volume. Yet, inconclusive evidence exists regarding the association of longer disease duration with decreased or increased regional GM volume in other chronic MSKP conditions.[37,67,97] Moreover, moderate evidence is present for a correlation between higher pain intensity and FA alterations in regional WM tracts[48,56,61,68] and FC alterations.[9,22,32,43,44,49,51,73,74,98] However, inconclusive evidence was found regarding the direction of the relation of pain intensity and pressure pain sensitivity with micro-structural WM[48,56,61,68] and FC[9,22,32,43,44,49,51,73,74,98] alterations in chronic MSKP.

It can be summarized that different chronic MSKP syndromes, which seem to be a heterogeneous group, expose unique (specific for each chronic MSKP condition) anatomical 'brain signatures' and functional reorganization. However, among all included chronic MSKP conditions it seems that brain regions involved in the limbic-affective and cognitive component of pain processing are involved in the observed neuroplastic brain remodeling. On the basis of this compelling evidence it can be stated that chronic MSKP is not only involved with somatosensory processing but also critically involves cognitive and affective-limbic processing in regions such as the ACC, insula, prefrontal cortex, and amygdala.

Important to discuss is that the observed relations (eg, extent and direction) between clinical pain characteristics and brain alterations can be influenced by multiple factors. Research has shown in chronic MSKP and non-MSKP patients the influence on pain and neuroplasticity of sex, age, genetics, environment, preexisting vulnerabilities, previous experiences, medication, culture, and psychosocial factors.[8,16,22,35,41,58,63,65,69,88,96] Accordingly, all of these variables could interfere with the observed relations between brain alterations and clinical pain measures and therefore may explain the incongruence found in this systematic review.

Various hypotheses can be made to explain the relation between clinical pain measures and GM decreases. It has been suggested that GM decrease is associated with long-term nociceptive input and neuroplastic changes.[5,50,84] Furthermore, increased cortical thickness for example in frontal brain regions could be the consequence of increased cognitive load in chronic pain conditions.[67] The frontal pole may process the cognitive dimension of pain, which suggests that pain has a cognitive load and this may require continuous engagement of regions in the frontal cortex and subsequently may lead to cortical thickening. The same theories could be hypothesized for alterations in limbic-affective brain regions.

To put the results of the current systematic review into a broader perspective, scientific studies regarding the relations between brain alterations and clinical pain measures in chronic non-MSKP patients should be reported. Research in other chronic pain syndromes such as irritable bowel syndrome and complex regional pain syndrome has also investigated the relationship between structural and functional brain alterations, and clinical pain measures such as pain intensity, pain inhibition, and pain duration.[12,79,91,94] Positive and negative correlations between clinical pain measures and GM morphology alterations have been reported in chronic non-MSKP patients in similar regions involved in somatosensory, affective, and cognitive components of pain processing, as reported in chronic MSKP patients.[12,79,91] The observed relations between brain alterations and clinical pain measures in chronic non-MSKP patients are in accordance with the results of our systematic review, but the direction of the relation was often conflicting.

Table 4 Summary of evidence regarding interrelations between brain alterations and clinical pain measures

Brain structural & functional alterations ~ Clinical pain measures	Strength of Conclusion	Reference
Interrelations between **GM alterations** and **clinical pain measures** in chronic MSKP		
↘ GM volume (ACC) ~ ↗ clinical pain intensity	Moderate evidence (2)	71, 96
↘ GM volume (pain processing regions) ~ ↗ pressure pain sensitivity	Moderate evidence (2)	22, 96
Δ in regional GM volume (pain processing regions) ~ ↗ pain duration	Moderate evidence (2)	37, 67, 96
↘ total GM volume ~ ↗ pain duration in FM	Preliminary evidence (3)	53
↘ GM thickness (pain processing regions) ~ ↗ pain intensity and unpleasantness	Preliminary evidence (3)	67
Interrelations between **WM alterations** and **clinical pain measures** in chronic MSKP		
Δ in FA (WM tracts involved in transmission of somatosensory, pain, affective, and cognitive information) ~ ↗ clinical pain intensity	Moderate evidence (2)	56, 48, 61, 68
↗ AD (WM tracts involved in transmission of information through the basal ganglia) ~ ↗ subjective pain scores	Preliminary evidence (3)	56
Interrelations between **FC alterations** and **clinical pain measures** in chronic MSKP		
Δ in FC (brain regions/networks involved in somatosensory, pain, and affect-cognitive processing of pain) ~ ↗ clinical pain intensity	Moderate evidence (2)	10, 22, 32, 44, 74, 43, 49, 51, 73, 98
Δ in FC (brain regions/networks involved in somatosensory, pain, and affect-cognitive processing of pain) ~ ↗ pressure pain sensitivity	Moderate evidence (2)	32, 43, 44
Interrelations between **functional activity alterations** and **clinical pain measures** in chronic MSKP		
↘ functional activity (temporal, occipital regions) ~ ↗ clinical pain intensity and allodynia	Preliminary evidence (3)	60
↗ functional activity in anterior insula ~ ↗ clinical pain intensity	Preliminary evidence (3)	28

Abbreviations: ↘ decreased; ↗, increased; FM, fibromyalgia.

Table 5 Glossary of important terms regarding MRI analysis of brain alterations

DMN	A constellation of brain regions thought to be involved in self-referential thinking.[18, 32] The DMN is deactivated during various externally focused task conditions.
ICA	Technique to analyse resting-state fMRI data, which allows for the estimation of resting-state or functional connectivity networks.
EAN	The frontoparietal *executive attention network* is a brain network involved in cognitive processing of working memory and attention.[23, 70]
FA	Fractional anisotropy is a measure of the degree of diffusion anisotropy. The FA is normalized so that it ranges from zero (diffusion is isotropic) to one (diffusion is constrained along one axis only). FA is typically much higher in WM structures than in CSF and GM, due to the highly organized and tightly packed myelinated axons in WM. Because of this, FA is often used as a surrogate marker for WM 'integrity'.[14]
AD	Axial diffusivity is a measure of diffusion along the first eigenvector. Decreased AD but unchanged radial diffusivity is typically assumed to indicate axonal damage or a lower axonal density.[14] As such, AD leads to a more specific interpretation of the concept of WM 'integrity' associated with FA.
Z-score	A Z-score is a way of standardizing the scale of two distributions. When the scales have been standardized, it is easier to compare scores on one distribution to scores on the other distribution. The mean of a distribution of Z-scores is always 0. The standard deviation of a distribution of Z-scores is always 1.
T-score	The T-score is a measure not of the strength of the association but the confidence with which we can assert that there is an association. A T-score is a standard score Z shifted and scaled to have a mean of 50 and a standard deviation of 10.

Abbreviations: DMN, default mode network; ICA, independent components analysis; EAN, executive attention network; fMRI, functional MRI; FA, fractional anisotropy; AD, axial diffusivity; GM, grey matter; WM, white matter; CSF, cerebrospinal fluid

Clinical Relevance and Implications

To our knowledge, this is the first systematic review summarizing the current evidence regarding relations between brain alterations explored with MRI, and clinical pain correlates (ie, pain duration, pain intensity, pain perception, pressure sensitivity, hyperalgesia, hypersensitivity, allodynia, and referred pain) in patients with chronic MSKP. Regarding the results, it can be stated that structural and functional brain alterations are closely related to clinical aspects of pain perception, modulation, and duration. Increased pain intensity and pressure pain sensitivity seem to be related to decreased GM volume in regions involved in somatosensory, affective, and cognitive processing of pain. In contrast, inconclusive evidence was found regarding the direction of the relation between WM and FC alterations, and increased pain intensity or pressure pain sensitivity.

On the basis of the summarized evidence, we can presume that central pain processing mechanisms of the brain play a crucial role in the persistent pain complaints of

patients with chronic MSKP. It is clear that pain is associated with a complex interplay among various brain regions and networks. Therefore, it can be recommended that the rehabilitation of patients with chronic MSKP has to be biopsychosocially-driven and that the central nervous system, including the brain has to be addressed. Recently, Baliki and Apkarian and colleagues concluded in 2 reviews that the activity and neuroplasticity of the limbic system plays a crucial role in the chronification of pain.[4,8] This statement is in accordance with our observations of alterations in brain regions that are often engaged in emotional, motivational, and cognitive processing of pain. However, on the basis of the current systematic review and on the available literature the causality of the relations between brain alterations and chronic pain is not yet clear.

Limitations and Strengths

When interpreting the results, the following study limitations have to be taken into account. First, 50% of included studies did not report visual inspection of the raw MRI data quality. Visual inspection of data quality is, however, extremely important in MRI research.[42] Nevertheless, all included studies adequately reported the application of manual exclusion in case of low data quality and/or included automated data adjustment in the pre-processing pipeline. The latter is equally important to obtain valid and reliable structural and fMRI data results.[46,86] Second, despite the fact that neuroimaging research in chronic pain conditions has not only shown alterations in brain structure[5] and function[44] but also alterations in brain chemistry,[33] we did not include articles on brain chemistry. In addition, studies using other functional neuroimaging techniques such as positron emission tomography, magnetoencephalography, and electroencephalography were not included. However, this was beyond the scope of this systematic review. Further, it is crucial to mention the fact that different MRI analytical techniques were used in the included studies, because the specific MRI acquisition and analytical technique can very much affect the outcome of a study. Next, it should be noted that the included studies used different standardized scales or questionnaires to measure clinical features of pain. This might result in difficulties comparing results of different studies. Last, when interpreting results of correlation analyses it is important to realize that a correlation between 2 variables does not imply a causal relationship. Therefore, no conclusions can be drawn on the causality of the observed relations. Longitudinal studies are required to unravel the direction of the relations and to answer the question of causality: Are brain alterations the result or the origin of chronic pain or a combination?

Several strengths of this systematic review can be outlined. First, the present study is innovative and has important clinical relevance. Second, the methodological quality of the included studies was moderate to good. Furthermore, the methods used for screening and scoring were completed by 2 independent blinded researchers. Last, the NOS was modified by adding 2 items specifically developed for the topic of the current systematic review. Consequently, the methodological quality and risk of bias of the brain MRI articles could have been evaluated more thoroughly giving a more accurate view on the preprocessing of the MRI analyses.

Recommendations for Further Research

In most included studies, the investigation of interrelations between brain alterations and clinical pain measures was a secondary aspect. Researchers are mostly primarily interested in the differences in brain structure and function between patients with pain and healthy individuals. In future research, it is important to include correlation analyses between clinical pain measures and brain alterations as a primary focus of interest. The current systematic review has not included studies that investigated the relation between brain alterations and pain measures associated with maladaptive pain cognitions such as pain catastrophizing and hypervigilance. Maladaptive pain cognitions could be interesting to include in a future systematic review on this topic.

Many chronic MSKP conditions remain largely unexplored regarding this research topic. It would be valuable in future research to investigate also patients with chronic whiplash and chronic idiopathic neck pain regarding brain alterations and the relation with clinical correlates of pain.

Furthermore, it could be interesting to explore the relation between brain alterations and experimental measures of central pain modulation, such as temporal summation or the efficacy of conditioned pain modulation. Efficacy of pain inhibition and the degree of bottom-up sensitization could then be related to potential brain alterations. The interaction between structural and functional brain alterations would also be a valuable research topic. In addition, it could be interesting to further examine the relationship between structural and functional brain connectivity in chronic MSKP patients. Innovative analytical techniques such as graph theoretical analyses of structural and functional brain networks (ie, connectomics) could be applied in pain research and can contribute to an increased insight in chronic pain. Also, new and more advanced data acquisition and analytic techniques such as multishell diffusion MRI and multitissue constrained spherical deconvolution should be used in future pain research.[45] Further, it will be a challenge for researchers and physicians to integrate brain neuroimaging including structural and fMRI into clinical/radiological practice at the individual level.

Finally, despite existing longitudinal brain research in patients with chronic MSKP[10,23,39,62,72,85] the causality of the relations between brain alterations and chronic pain is not yet elucidated. Further research is warranted to clarify the direction of the relations of structural and functional brain alterations with clinical pain measures. It remains a crucial issue to further explore the underlying mechanisms of pain chronification and the role of brain alterations in this transition. Last, it will be a challenge for researchers to explore the effectiveness of different therapy strategies for chronic MSKP patients by analyzing the effects of specific interventions on brain morphological and functional alterations as well as on clinical measures of pain using a longitudinal design.

Conclusion

Moderate evidence was found for relations between clinical pain measures and structural, regional GM and WM morphology, and FC brain alterations within regions involved in somatosensory, affective-motivational, and cognitive processing of pain in chronic MSKP patients. Nevertheless, inconclusive results were found regarding the direction of these relations. Further research is warranted to unravel whether these brain alterations occur as a result of chronic pain or vice versa and whether these alterations are positively or negatively related to clinical measures of pain.

Supplementary data

Supplementary Table 1 Level of evidence, according to the 2005 classification system of the Dutch Institute for Healthcare Improvement CBO

	Intervention
A1	Systematic review of at least 2 independent from each other conducted studies of evidence level A2
A2	Randomized double-blinded comparative clinical research of good quality and efficient size
B	Comparative research, but not with al characteristics as mentioned for A2. This includes also patient-control research and cohort research.
C	Not comparative research
D	Opinion of experts

www.cbo.nl

Supplementary Table 2 Strength of Conclusion (modified table)

	Conclusion based on
1	Research of evidence level A1 or at least 2 independent conducted studies of evidence level A2
2	1 research of evidence level A2 or at least 2 independent conducted studies of evidence level B
3	1 research of evidence level B or C
4	Opinion of experts or Inconclusive or inconsistent results between various studies

Supplementary Table 3

Studies	Chronic MSK pain patients (n)	Healthy controls (n)	Measurement techniques: Brain MRI	Measurement techniques: Clinical pain measures	Correlations, relations, associations	Main results in chronic MSK pain patients	Correlation coefficient (r), T-score, Z-score, p-value
Structural MRI techniques							
Gray matter morphology							
Kuchinad et al. (2007)[53]	FM (10) ♀	HC (10) ♀ Age-matched	MRI (T1) VBM	Disease duration	**Disease duration** ~ Total GMV	- Correlation: 1. ↗disease duration ~ ↘total GMV	r= - 0.79 p= 0.007
Younger et al. (2010)[96]	M-TMD (14) ♀ Bilateral pain	HC (15) ♀ Age-matched	MRI (T1) VBM	Disease duration, NRS (current pain), Algometry (pressure pain sensitivity)	**Disease duration**, **Pain intensity, Pressure pain sensitivity,** ~ regional GMV	+ Correlation: 1. ↗Disease duration ~ ↗GMV R&L PCC, R hippocampus, R&L midbrain & R mid cerebellar peduncle - Correlation: 2. ↗pain intensity ~ ↘GMV in R rostral ACC, R PCC with precuneus, SFG & STG 3. ↗pressure pain sensitivity ~ ↘GMV R&L trigeminal nuclei	T-scores: 1. 4.78-5.63 2. 5.58-5.93 3. 5.02-8.00
Gerstner et al. (2011)[5]	M-TMD (9) ♀ R-handed	HC (9): ♀ Age-matched R-handed	MRI (T1) VBM	Pain duration	**Pain duration** ~ regional GMV	- Correlation: 1. ↗pain duration ~ ↘GMV R STG/MTG	r= - 0.78 p<0.05
Moayedi et al. (2011)[67]	M-TMD (17) ♀ Idiopathic R-handed	HC (17) ♀ R-handed	MRI (T1) VBM Cortical thickness analysis	NRS (current pain), NRS (pain unpleasantness), NRS (average pain & unpleasantness last month), Disease duration	**Pain intensity,** **Pain unpleasantness** ~ GM thickness **Disease duration** ~ regional GMV	- Correlation: 1. ↗pain intensity ~ ↘GM thickness in ant MCC & ventro-lateral M1 2. ↗pain unpleasantness ~ ↘GM thickness in L OFC + Correlation: 3. ↗disease duration ~ ↗GMV in the sensory thalamus	1. r= - 0.83 p<0.05 2. r= - 0.74 p<0.05 3. r= 0.91 p<0.05
Mordasini et al. (2012)[68]	CPPS (20) ♂ Refractory Idiopathic R-handed	HC (20) ♂ Age-matched R-handed	MRI (T1) VBM	NIH-CPSI (pain subscale)	**Pain intensity** ~ regional GMV	- Correlation: 1. ↗pain intensity ~ ↘GMV L ACC	1. r= - 0.51 p= 0.02
White matter properties							
Lutz et al. (2008)[61]	FM (30) ♀ R-handed	HC (30) ♀ Age-matched R-handed	MRI (T1, T2) DTI	Pain intensity (FIQ subscore for pain)	**Pain intensity** ~ FA of regional WM	+ Correlation: 1. ↗pain intensity ~ ↗FA in the WM of the R superior frontal gyrus	1. r= 0.43 p= 0.03
Kim et al. (2014)[48]	FM (19) ♀ R-handed	HC (18) ♀ Age-matched R-handed	MRI (T1, T2) DTI	VAS SF-MPQ (sensory pain subscore)	**Pain intensity** ~ FA of regional WM	- Correlation: 1. ↗sensory pain score ~ ↘FA in corpus callosum body	1. r= - 0.47 p= 0.04

Supplementary Table 3 Continued

Studies	Chronic MSK pain patients (n)	Healthy controls (n)	Measurement techniques: Brain MRI	Measurement techniques: Clinical pain measures	Correlations, relations, associations	Main results in chronic MSK pain patients	Correlation coefficient (r), T-score, Z-score, p-value
Structural MRI techniques							
White matter properties							
Moayedi et al. (2012)[68]	M-TMD (17) ♀ Idiopathic R-handed	HC (17) ♀ R-handed	DTI	NRS (current pain), NRS (pain unpleasantness)	**Pain intensity**, **Pain unpleasantness** ~ FA of regional WM	- Correlation: 1. ↗ Pain intensity ~ ↘ FA in internal & ext/ extreme capsules 2. ↗ Pain intensity ~ ↘ FA in thalamic cluster 3. ↗ Pain intensity ~ ↘ FA in R internal capsule 4. ↗ pain unpleasantness ~ ↘ FA in R internal capsule	1. r= - 0.49 p= 0.046 2. r= - 0.59 p= 0.013 3. r= - 0.72 p= 0.001 4. r= - 0.63 p= 0.007
Lieberman et al. (2014)[56]	Chronic MSK (46) ♂ (12) ♀ (34)	HC (33) ♂ (14) ♀ (19) Age-matched	DTI	Pain severity, SF-MPQ, Pain subscale of the TOPS	**Pain intensity** ~ FA & AD in regional WM	+ Correlation: 1. ↗ Total pain experience score ~ ↗ FA in the L uncinate fasciculus 2. ↗ Total pain experience score ~ ↗ AD in the L ant limb of the internal capsule 3. ↗ Total pain experience score ~ ↗ AD in the L posterior limb of the internal capsule 4. ↗ MPQ typical pain ~ ↗ FA in the L uncinate 5. ↗ MPQ typical pain ~ ↗ AD in the L anterior limb	1. r= 0.393 p= 0.007 2. r= 0.398 p= 0.006 3. r= 0.373 p= 0.011 4. r= 0.480 p= 0.001 5. r= 0.451 p= 0.002
Functional MRI techniques							
Napadow et al. (2010)[74]	FM (18) ♀ R-handed	HC (18) ♀ Age-matched R-handed	rs-fMRI *dual-regression probalistic ICA*	VAS (pain intensity immediately prior to rs-fMRI scan)	**Pain intensity** ~ FC	+ Covariation: 1. ↗ pain intensity ~ ↗ FC: a. DMN to (R mid & ant IC, cerebellum, dlPFC & sgACC) b. R EAN to (R ant & L mid & post IC & putamen) - Covariation: 2. ↗ pain intensity ~ ↘ FC: 1. R EAN to (Hippocampus, PAG, nucleus cuneiformis & pontine raphe)	Z-scores 1. DMN – ant IC: Low pain: -1.72(+/- 0.81) High pain: 0.55(+/- 1.32) R EAN: Low pain: 0.57(+/- 0.48) High pain: 2.72(+/- 0.95) 2. R EAN – PAG Low pain: 1.45(+/-0.91) High Pain: -0.52(+/-1.68)
Flodin et al. (2014)[32]	FM (16) ♀	HC (22) ♀ Age-matched	*MRI (T1)* rs-fMRI fMRI (pain exposure)	VAS, Computer-controlled pressure stimulator	**Pressure pain sensitivity** ~ FC	+ Correlation: 1. ↗pressure pain sensitivity ~ ↗FC - L IC & dorsal PCC - L rolandic operculum & L parahippocampus - Thalamus & PFC	+ correlation p<0.00031

Supplementary Table 3 Continued

Studies	Chronic MSK pain patients (n)	Healthy controls (n)	Measurement techniques		Correlations, relations, associations	Main results in chronic MSK pain patients	Correlation coefficient (r), T-score, Z-score, p-value
			Brain MRI	Clinical pain measures			
Functional MRI techniques							
Ichesco et al. (2014)[44]	FM (18) ♀ R-handed	HC (18) ♀ Age-matched R-handed	*MRI (T1)* rs-fMRI: fMRI: (experimental pain)	PRI, GBS, Pressure stimulator	**PPT** ~ IC-CC connectivity **Clinical pain** ~ IC connectivity	+ Correlation: 1. ↗Clinical pain ~ ↗FC: - R ant IC & STG 2. ↗Affective scores PRI ~ ↗FC: - IC & STG 3. ↗Sensory scores PRI ~ ↗FC - R mid IC & L&R precuneus	1. r= 0.51 p=0.03 2. Z= 3.79 p<0.001 3. Z= 4.48 p<0.001
López-Solà et al. (2014)[60]	FM (35)	HC (25) Age-matched handedness -matched	fMRI	NRS, Non-nociceptive sensory stimulation (auditory, visual, tactile stimulation): allodynia, Sensory hypersensitivity	**Pain intensity**, **Sensory hypersensitivity** ~ Brain activity	- Correlation: 1. ↗pain intensity ~ ↘activation in visual brain areas 2. ↗hypersensitivity to tactile stimulation (allodynia) ~ ↘activation in superior/ middle temporal gyri	T-scores: 1. lat occ cortex: -3.92 Med occ cortex: - 3.69 2. Sup temporal gyri: - 3.79; 3.00
Kim et al. (2015)[49]	FM (35) ♀(32) ♂(3)	HC (14) ♀(10) ♂(4) Age-matched	rs-fMRI fMRI (6-minute continuous pain-state run)	NRS, BPI, Pressure-pain stimuli (cuff algometry)	**Pain intensity,** **TS of pain** ~ FC	+ Correlation: 1. ↗clinical pain intensity at time of rs-fMRI scan ~ ↗changes (from the pain phase through the rest phase) in S1leg connectivity to the Ant INS 2. ↗TS of pain ~ ↗changes in S1leg connectivity to R Ant/Mid INS - Correlation: 3. ↗clinical pain intensity at time of rs-fMRI scan ~ ↘resting connectivity within S1	1. r= 0.51 2. r= 0.37 3. T= - 12.30 p<0.001
Kong et al. (2013)[51]	CLBP (18) ♂(6) ♀(12)	HC (18) ♂(6) ♀(12) Age-, sex- & race-matched	MRI (T1) rs-fMRI	VAS, Pain-inducing exercises	**VAS changes** ~ FC	+ Correlation 1. ↗pain intensity change after exercises ~ ↗FC at the L insula, precuneus, amygdala & fusiform	Z-scores: Insula: 3.8 Precuneus: 3.93 Amygdala: 2.86 Fusiform: 3.23
Yu et al. (2014)[98]	CLBP (18) ♂(6) ♀(12)	HC (18) ♂(6) ♀(12) Age-, sex- & race-matched	MRI (T1) rs-fMRI	VAS , BPI, Pain-inducing exercises	**Pain intensity**, **Pain duration** ~ FC	- Correlation 1. High pain: ↗LBP ratings ~ ↘FC between PAG & L vmPFC (after exc) - Correlation 2. ↗disease duration ~ ↘FC between PAG & R posterior insula & PAG-L amygdala (before exc)	Z-scores: 1. PAG-L vmPFC: 2.96 2. R posterior inula-L amygdala: 3.04
Baliki et al. (2014)[10]	CLBP (18) ♂(13) ♀(5) OA (14): ♂(8) ♀(6)	HC (36) ♂(12) ♀(24) Right-handed	MRI (T1) VBM rs-fMRI	Pain intensity, Pain duration	**Pain intensity** ~ FC **Pain duration** ~ HF power within the DMN, phase differences	CLBP, OA: + Correlation 1. ↗pain intensity at the time of the scan ~ ↗MPFC-INS connectivity 2. ↗High frequency power within the DMN ~ ↗pain duration. CLBP: 3. ↗phase differences between the DMN and frontoparietal network ~ ↗pain duration.	CLBP: 1. r= 0.75 p<0.01 2. r= 0.65 p<0.01 3. r= 0.68 p<0.01 OA: 1. r= 0.61 p<0.05 2. r= 0.77 p<0.01

Supplementary Table 3 Continued

Studies	Chronic MSK pain patients (n)	Healthy controls (n)	Measurement techniques		Correlations, relations, associations	Main results in chronic MSK pain patients	Correlation coefficient (r), T-score, Z-score, p-value
			Brain MRI	Clinical pain measures			
Functional MRI techniques							
Ichesco et al. (2012)[43]	M-TMD (8) ♀	HC (8) ♀ Age-matched	MRI (T1) rs-fMRI fMRI (pressure pain stimuli)	VAS, PRI (SF-MPQ), BPI	**Pain intensity, Experimental pressure pain** ~ FC	- Correlation: 1. ↗ pain intensity ~ ↘ L anterior IC-rostral ACC connectivity 2. ↗ MPQ total scores ~ ↘ L anterior IC-rostral ACC connectivity 3. ↗ pressure sensitivity ~ ↘ FC between L ant IC & rostral ACC/the medial frontal gyrus	1. r= - 0.952 p<0.001 2. r= - 0.830 p<0.011 3. r= - 0.838 p<0.009
Structural & Functional MRI techniques							
Ceko et al. (2013)[22]	Younger FM (14) Older FM (14) ♀	Younger HC (15) Older HC (13) ♀ Age-matched handedness – matched	MRI (T1) VBM rs-fMRI	NRS (Pain intensity) Pain duration Experimental pressure pain sensitivity	**Pressure pain sensitivity** ~ regional GMV **Pain intensity**, **Pain duration** ~ FC	Younger FM patients: - Correlation: 1. ↗ unpleasantness ratings of the pressure stimuli ~ ↘ GMV in LaINS 2. ↗ pressure pain sensitivity ~ ↘ GMV in NAc + Correlation: 3. ↗ pain intensity during rs-fMRI ~ ↗ connectivity of LaINS to S1 & M1 4. ↗ pain duration ~ ↗ connectivity of LaINS to S1 & M1Older FM patients: - Correlation: 5. ↗ pressure pain sensitivity ~ ↘ GMV in PCC	1. T= 12.14 p<0.001 2. T= 6.06 p= 0.001 3. T= 6.06 p= 0.001 4. r=0.54 p= 0.047 5. T= 5.32 p= 0.014
Farmer et al. (2011)[28]	CPPS (19) ♂ Right-handed	HC (16) Age- & sex-matched	MRI (T1) fMRI (pain task & visual task) DTI	VAS, MPQ, Pain duration	**Pain intensity** ~ brain activity & regional GM density **Pain duration** ~ regional GM density	+ Correlation: 1. ↗ pain intensity ~ ↗ Ant INS activity 2. ↗ MPQ score ~ ↗ Ant INS activity 3. ↗ pain intensity ~ ↗ Ant INS GM density 4. ↗ pain duration ~ ↗ ACC GM density	1. r= 0.57 p<0.05 2. Z=0.78 p<0.01 3. r= 0.69 p<0.01 4. r= 0.69 p<0.01

R = Right; L = Left; OA = Osteoarthritis; HC = Healthy Controls; MRI = Magnetic Resonance Imaging; VBM = Voxel-based Morphometry; VAS = Visual Analog Scale; GMV= Grey Matter Volume; CPPS = Chronic Pelvic Pain Syndrome; NIH-CPSI = National Institutes of Health Chronic Prostatitis Symptom Index; ACC = Anterior Cingulate Cortex; M-TMD = Myofascial Temporomandibular disorder; STG = Superior Temporal Gyrus; MTG = Middle Temporal Gyrus; NRS = Numerical Rating Scale; PCC = Posterior Cingulate Cortex; SFG= Superior Frontal Gyrus; Mid = Middle; FM = Fibromyalgia; DTI = Diffusion Tensor Imaging; SF-MPQ = Short Form of the McGill Pain Questionnaire; FA = Fractional Anisotropy; TMD = Temporomandibular Disorder; EC/ExC= External/ Extreme Capsules; fMRI = Functional Magnetic Resonance Imaging; FC = Functional Connectivity; DMN = Default Mode Network; Ant = Anterior; IC = Insular Cortex; EAN = Executive Attention Network; Post = Posterior; dlPFC = Dorsolateral Prefrontal Cortex; sgACC = Subgenual Anterior Cingulate Cortices; PAG = Periaqueductal Grey; PRI = Pain rating Index; BPI = Brief Pain Inventory; GBS = Gracely Box Scale; NAc= nucleus accumbens; CC = Cingulate Cortex; MFG = Medial Frontal Gyrus; PFC = Prefrontal Cortex; Inf = Inferior; PPT = Pressure-pain Thresholds; CLBP = Chronic Low Back Pain; S1 = Primary Somatosensory Cortex; S2 = Secondary Somatosensory Cortex; IPL = Inferior Parietal Lobule; MSK = musculoskeletal; vmPFC = ventral medial prefrontal cortex ; CPPS= Chronic pelvic pain syndrome; FM= Fibromyalgia; MSK= musculoskeletal pain; MPQ= McGill Pain Questionnaire; BPI= Brief Pain Inventory; TS= Temporal Summation; aINS= anterior Insula; TOPS= Treatment Outcomes in Pain Survey; IC= insular cortex; PRI= pain rating index; MCC= mid-cingulate cortex; OFC= orbitofrontal cortex; M-TMD= myofascial pain of the masticatory region; M1=primary motor cortex; FIQ= Fibromyalgia Impact Questionnaire; exc=excercises; ICA= independent components analysis

Disclosures

Iris Coppieters and Dorien Goubert, PhD students at University Ghent, are funded by the Special Research Fund of Ghent University (BOF-Ghent). Jeroen Kregel, PhD student at University Ghent, is funded by the agency for innovation by Science and Technology in Flanders (IWT-Brussels, Belgium).

References

1. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. Neurotherapeutics. 4:316-29, 2007.
2. Apkarian AV. Frontiers in Neuroscience Human Brain Imaging Studies of Chronic Pain: Translational Opportunities. In: Kruger L, Light AR, editors. Translational Pain Research: From Mouse to Man. Boca Raton, FL: CRC Press Llc.; 2010.
3. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. Pain. 152:S49-64, 2011.
4. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain. 9:463-84, 2005.
5. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J Neurosci. 24:10410-5, 2004.
6. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage. 11:805-21, 2000.
7. Assaf Y, Pasternak O. Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. J Mol Neurosci. 34:51-61, 2008.
8. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. Neuron. 87:474-91, 2015.
9. Baliki MN, Schnitzer TJ, Bauer WR, Apkarian AV. Brain morphological signatures for chronic pain. PLoS One. 6:e26010, 2011.
10. Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional reorganization of the default mode network across chronic pain conditions. PLoS One. 9:e106133, 2014.
11. Baliki MN, Petre B, Torbey S, Herrmann KM, Huang L, Schnitzer TJ, Fields HL, Apkarian AV. Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci. 15:1117-9, 2012.
12. Barad MJ, Ueno T, Younger J, Chatterjee N, Mackey S. Complex regional pain syndrome is associated with structural abnormalities in pain-related regions of the human brain. J Pain. 15:197-203, 2014.
13. Barkhof F, Haller S, Rombouts SA. Resting-state functional MR imaging: a new window to the brain. Radiology. 272:29-49, 2014.
14. Barsky AJ, Borus JF. Functional somatic syndromes. Ann Intern Med. 130:910-21, 1999.
15. Belliveau JW, Kwong KK, Kennedy DN, Baker JR, Stern CE, Benson R, Chesler DA, Weisskoff RM, Cohen MS, Tootell RB, Fox PT, Brady TJ, Rosen BR. Magnetic resonance imaging mapping of brain function. Human visual cortex. Invest Radiol. 27 Suppl 2:S59-65, 1992.
16. Blankstein U, Chen J, Diamant NE, Davis KD. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. Gastroenterology. 138:1783-9, 2010.
17. Bourke JH, Langford RM, White PD. The common link between functional somatic syndromes may be central sensitisation. J Psychosom Res. 78:228-36, 2015.
18. Buckner RL, Krienen FM, Yeo BT. Opportunities and limitations of intrinsic functional connectivity MRI. Nat Neurosci. 16:832-7, 2013.
19. Bushnell MC, Ceko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci. 14:502-11, 2013.
20. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. Semin Arthritis Rheum. 44:68-75, 2014.
21. Cauda F, Palermo S, Costa T, Torta R, Duca S, Vercelli U, Geminiani G, Torta DM. Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. Neuroimage Clin. 4:676-86, 2014.
22. Ceko M, Bushnell MC, Fitzcharles MA, Schweinhardt P. Fibromyalgia interacts with age to change the brain. Neuroimage Clin. 3:249-60, 2013.
23. Ceko M, Shir Y, Ouellet JA, Ware MA, Stone LS, Seminowicz DA. Partial recovery of abnormal insula and dorsolateral prefrontal connectivity to cognitive networks in chronic low back pain after treatment. Hum Brain Mapp. 36:2075-92, 2015.
24. Cimmino MA, Ferrone C, Cutolo M. Epidemiology of chronic musculoskeletal pain. Best Pract Res Clin Rheumatol. 25:173-83, 2011.
25. Correa JB, Costa LO, de Oliveira NT, Sluka KA, Liebano RE. Central sensitization and changes in conditioned pain modulation in people with chronic nonspecific low back pain: a case-control study. Exp Brain Res. 233:2391-9, 2015.

26. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. Journal of Neuroimmune Pharmacology. 8:518-534, 2013.
27. Denk F, McMahon SB, Tracey I. Pain vulnerability: a neurobiological perspective. Nat Neurosci. 17:192-200, 2014.
28. Farmer MA, Chanda ML, Parks EL, Baliki MN, Apkarian AV, Schaeffer AJ. Brain functional and anatomical changes in chronic prostatitis/chronic pelvic pain syndrome. J Urol. 186:117-24, 2011.
29. Fernandez-de-las-Penas C, Galan-del-Rio F, Fernandez-Carnero J, Pesquera J, Arendt-Nielsen L, Svensson P. Bilateral widespread mechanical pain sensitivity in women with myofascial temporomandibular disorder: evidence of impairment in central nociceptive processing. J Pain. 10:1170-8, 2009.
30. Fischl B. FreeSurfer. Neuroimage. 62:774-81, 2012.
31. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. Neuroimage. 9:195-207, 1999.
32. Flodin P, Martinsen S, Lofgren M, Bileviciute-Ljungar I, Kosek E, Fransson P. Fibromyalgia is associated with decreased connectivity between pain- and sensorimotor brain areas. Brain Connect. 4:587-94, 2014.
33. Foerster BR, Petrou M, Edden RA, Sundgren PC, Schmidt-Wilcke T, Lowe SE, Harte SE, Clauw DJ, Harris RE. Reduced insular gamma-aminobutyric acid in fibromyalgia. Arthritis Rheum. 64:579-83, 2012.
34. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 8:700-11, 2007.
35. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. Neuron. 60:570-81, 2008.
36. Geisser ME, Gracely RH, Giesecke T, Petzke FW, Williams DA, Clauw DJ. The association between experimental and clinical pain measures among persons with fibromyalgia and chronic fatigue syndrome. Eur J Pain. 11:202-7, 2007.
37. Gerstner G, Ichesco E, Quintero A, Schmidt-Wilcke T. Changes in regional gray and white matter volume in patients with myofascial-type temporomandibular disorders: a voxel-based morphometry study. J Orofac Pain. 25:99-106, 2011.
38. Gusnard DA, Raichle ME, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci. 2:685-94, 2001.
39. Hashmi JA, Baliki MN, Huang L, Baria AT, Torbey S, Hermann KM, Schnitzer TJ, Apkarian AV. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. Brain. 136:2751-68, 2013.
40. Hemington KS, Wu Q, Kucyi A, Inman RD, Davis KD. Abnormal cross-network functional connectivity in chronic pain and its association with clinical symptoms. Brain Struct Funct. 2015.
41. Henderson LA, Gandevia SC, Macefield VG. Gender differences in brain activity evoked by muscle and cutaneous pain: a retrospective study of single-trial fMRI data. Neuroimage. 39:1867-76, 2008.
42. Herbst M, Maclaren J, Lovell-Smith C, Sostheim R, Egger K, Harloff A, Korvink J, Hennig J, Zaitsev M. Reproduction of motion artifacts for performance analysis of prospective motion correction in MRI. Magn Reson Med. 71:182-90, 2014.
43. Ichesco E, Quintero A, Clauw DJ, Peltier S, Sundgren PM, Gerstner GE, Schmidt-Wilcke T. Altered functional connectivity between the insula and the cingulate cortex in patients with temporomandibular disorder: a pilot study. Headache. 52:441-54, 2012.
44. Ichesco E, Schmidt-Wilcke T, Bhavsar R, Clauw DJ, Peltier SJ, Kim J, Napadow V, Hampson JP, Kairys AE, Williams DA, Harris RE. Altered resting state connectivity of the insular cortex in individuals with fibromyalgia. J Pain. 15:815-826.e1, 2014.
45. Jeurissen B, Tournier JD, Dhollander T, Connelly A, Sijbers J. Multi-tissue constrained spherical deconvolution for improved analysis of multi-shell diffusion MRI data. Neuroimage. 103:411-26, 2014.
46. Jones DK, Cercignani M. Twenty-five pitfalls in the analysis of diffusion MRI data. NMR Biomed. 23:803-20, 2010.
47. Jones SE, Buchbinder BR, Aharon I. Three-dimensional mapping of cortical thickness using Laplace's equation. Hum Brain Mapp. 11:12-32, 2000.
48. Kim DJ, Lim M, Kim JS, Son KM, Kim HA, Chung CK. Altered white matter integrity in the corpus callosum in fibromyalgia patients identified by tract-based spatial statistical analysis. Arthritis Rheumatol. 66:3190-9, 2014.
49. Kim J, Loggia ML, Cahalan CM, Harris RE, Beissner F, Garcia RG, Kim H, Barbieri R, Wasan AD, Edwards RR, Napadow V. The somatosensory link in fibromyalgia: functional connectivity of the primary somatosensory cortex is altered by sustained pain and is associated with clinical/autonomic dysfunction. Arthritis Rheumatol. 67:1395-405, 2015.
50. Kim JH, Suh SI, Seol HY, Oh K, Seo WK, Yu SW, Park KW, Koh SB. Regional grey matter changes in patients with migraine: a voxel-based morphometry study. Cephalalgia. 28:598-604, 2008.
51. Kong J, Spaeth RB, Wey HY, Cheetham A, Cook AH, Jensen K, Tan Y, Liu H, Wang D, Loggia ML, Napadow V, Smoller JW, Wasan AD, Gollub RL. S1 is associated with chronic low back pain: a functional and structural MRI study. Mol Pain. 9:43, 2013.
52. Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B. Structural and functional brain abnormalities in chronic low back pain: A systematic review. Semin Arthritis Rheum. 2015.
53. Kuchinad A, Schweinhardt P, Seminowicz DA, Wood PB, Chizh BA, Bushnell MC. Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? J Neurosci. 27:4004-7, 2007.
54. Kucyi A, Davis KD. The dynamic pain connectome. Trends Neurosci. 38:86-95, 2015.
55. Kucyi A, Moayedi M, Weissman-Fogel I, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Enhanced medial prefrontal-default mode network functional connectivity in chronic pain and its association with pain rumination. J Neurosci. 34:3969-75, 2014.
56. Lieberman G, Shpaner M, Watts R, Andrews T, Filippi CG, Davis M, Naylor MR. White Matter Involvement in Chronic Musculoskeletal Pain. Journal of Pain. 15:1110-1119, 2014.
57. Litcher-Kelly L, Martino SA, Broderick JE, Stone AA. A systematic review of measures used to assess chronic musculoskeletal pain in clinical and randomized controlled clinical trials. J Pain. 8:906-13, 2007.
58. Loggia ML, Berna C, Kim J, Cahalan CM, Martel MO, Gollub RL, Wasan AD, Napadow V, Edwards RR. The lateral prefrontal cortex mediates the hyperalgesic effects of negative cognitions in chronic pain patients. J Pain. 16:692-9, 2015.
59. Logothetis NK. What we can do and what we cannot do with fMRI. Nature. 453:869-78, 2008.
60. Lopez-Sola M, Pujol J, Wager TD, Garcia-Fontanals A, Blanco-Hinojo L, Garcia-Blanco S, Poca-Dias V, Harrison BJ, Contreras-Rodriguez O, Monfort J, Garcia-Fructuoso F, Deus J. Altered functional magnetic resonance imaging responses to nonpainful sensory stimulation in fibromyalgia patients. Arthritis Rheumatol. 66:3200-9, 2014.
61. Lutz J, Jager L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. Arthritis Rheum. 58:3960-9, 2008.
62. Mansour AR, Baliki MN, Huang L, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Brain white matter structural properties predict transition to chronic pain. Pain. 154:2160-8, 2013.
63. Martinez-Jauand M, Sitges C, Rodriguez V, Picornell A, Ramon M, Buskila D, Montoya P. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. Eur J Pain. 17:16-27, 2013.
64. Martucci KT, Shirer WR, Bagarinao E, Johnson KA, Farmer MA, Labus JS, Apkarian AV, Deutsch G, Harris RE, Mayer EA, Clauw DJ, Greicius MD, Mackey SC. The posterior medial cortex in urologic chronic pelvic pain syndrome: detachment from default mode network-a resting-state study from the MAPP Research Network. Pain. 156:1755-64, 2015.
65. Matsuzawa-Yanagida K, Narita M, Nakajima M, Kuzumaki N, Niikura K, Nozaki H, Takagi T, Tamai E, Hareyama N, Terada M, Yamazaki M, Suzuki T. Usefulness of antidepressants for improving the neuropathic pain-like state and pain-induced anxiety through actions at different brain sites. Neuropsychopharmacology. 33:1952-65, 2008.
66. Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. Clin Rheumatol. 26:465-73, 2007.
67. Moayedi M, Weissman-Fogel I, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. Neuroimage. 55:277-86, 2011.
68. Moayedi M, Weissman-Fogel I, Salomons TV, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. White matter brain and trigeminal nerve abnormalities in temporomandibular disorder. Pain. 153:1467-77, 2012.
69. Mogil JS, Bailey AL. Sex and gender differences in pain and analgesia. Prog Brain Res. 186:141-57, 2010.
70. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 8:336-41, 2010.

71. Mordasini L, Weisstanner C, Rummel C, Thalmann GN, Verma RK, Wiest R, Kessler TM. Chronic pelvic pain syndrome in men is associated with reduction of relative gray matter volume in the anterior cingulate cortex compared to healthy controls. J Urol. 188:2233-7, 2012.
72. Mutso AA, Petre B, Huang L, Baliki MN, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Reorganization of hippocampal functional connectivity with transition to chronic back pain. J Neurophysiol. 111:1065-76, 2014.
73. Napadow V, Kim J, Clauw DJ, Harris RE. Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. Arthritis Rheum. 64:2398-403, 2012.
74. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis Rheum. 62:2545-55, 2010.
75. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. Man Ther. 15:135-41, 2010.
76. Nijs J, Meeus M, Van Oosterwijck J, Ickmans K, Moorkens G, Hans G, De Clerck LS. In the mind or in the brain? Scientific evidence for central sensitisation in chronic fatigue syndrome. Eur J Clin Invest. 42:203-12, 2012.
77. Nijs J, Torres-Cueco R, van Wilgen CP, Girbes EL, Struyf F, Roussel N, van Oosterwijck J, Daenen L, Kuppens K, Vanwerweeen L, Hermans L, Beckwee D, Voogt L, Clark J, Moloney N, Meeus M. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. Pain Physician. 17:447-57, 2014.
78. Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A. 87:9868-72, 1990.
79. Piche M, Chen JI, Roy M, Poitras P, Bouin M, Rainville P. Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls. J Pain. 14:1217-26, 2013.
80. Roussel NA, Nijs J, Meeus M, Mylius V, Fayt C, Oostendorp R. Central sensitization and altered central pain processing in chronic low back pain: fact or myth? Clin J Pain. 29:625-38, 2013.
81. Sarlani E, Greenspan JD. Evidence for generalized hyperalgesia in temporomandibular disorders patients. Pain. 102:221-6, 2003.
82. Schmidt-Wilcke T. Neuroimaging of chronic pain. Best Pract Res Clin Rheumatol. 29:29-41, 2015.
83. Schmidt-Wilcke T, Leinisch E, Ganssbauer S, Draganski B, Bogdahn U, Altmeppen J, May A. Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. Pain. 125:89-97, 2006.
84. Schmidt-Wilcke T, Leinisch E, Straube A, Kampfe N, Draganski B, Diener HC, Bogdahn U, May A. Gray matter decrease in patients with chronic tension type headache. Neurology. 65:1483-6, 2005.
85. Seminowicz DA, Shpaner M, Keaser ML, Krauthamer GM, Mantegna J, Dumas JA, Newhouse PA, Filippi CG, Keefe FJ, Naylor MR. Cognitive-behavioral therapy increases prefrontal cortex gray matter in patients with chronic pain. J Pain. 14:1573-84, 2013.
86. Shirer WR, Jiang H, Price CM, Ng B, Greicius MD. Optimization of rs-fMRI Pre-processing for Enhanced Signal-Noise Separation, Test-Retest Reliability, and Group Discrimination. Neuroimage. 117:67-79, 2015.
87. Tournier JD, Mori S, Leemans A. Diffusion tensor imaging and beyond. Magn Reson Med. 65:1532-56, 2011.
88. Tsang A, Von Korff M, Lee S, Alonso J, Karam E, Angermeyer MC, Borges GL, Bromet EJ, Demytteneare K, de Girolamo G, de Graaf R, Gureje O, Lepine JP, Haro JM, Levinson D, Oakley Browne MA, Posada-Villa J, Seedat S, Watanabe M. Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. J Pain. 9:883-91, 2008.
89. Tuch DS, Reese TG, Wiegell MR, Makris N, Belliveau JW, Wedeen VJ. High angular resolution diffusion imaging reveals intravoxel white matter fiber heterogeneity. Magn Reson Med. 48:577-82, 2002.
90. Tuzun EH. Quality of life in chronic musculoskeletal pain. Best Pract Res Clin Rheumatol. 21:567-79, 2007.
91. Valet M, Gundel H, Sprenger T, Sorg C, Muhlau M, Zimmer C, Henningsen P, Tolle TR. Patients with pain disorder show gray-matter loss in pain-processing structures: a voxel-based morphometric study. Psychosom Med. 71:49-56, 2009.
92. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. Eur J Pain. 17:299-312, 2013.
93. Wells G SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. 2014. Available from: http://www.ohri.ca/programs/clinical_epidemiology/ oxford.asp.
94. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. Gut. 53:1595-601, 2004.
95. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ. 81:646-56, 2003.
96. Younger JW, Shen YF, Goddard G, Mackey SC. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. Pain. 149:222-8, 2010.
97. Younger JW, Chu LF, D'Arcy NT, Trott KE, Jastrzab LE, Mackey SC. Prescription opioid analgesics rapidly change the human brain. Pain. 152:1803-10, 2011.
98. Yu R, Gollub RL, Spaeth R, Napadow V, Wasan A, Kong J. Disrupted functional connectivity of the periaqueductal gray in chronic low back pain. Neuroimage Clin. 6:100-8, 2014.
99. Zeng X, Zhang Y, Kwong JS, Zhang C, Li S, Sun F, Niu Y, Du L. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. J Evid Based Med. 8:2-10, 2015.

Chapter 2

Is traumatic and non-traumatic neck pain associated with brain alterations? A systematic review

De Pauw R. MSc[1,*], Coppieters I. MSc[1, 2,*] ,Meeus M. PhD[1, 2, 3], Caeyenberghs K. PhD [4], Danneels L. PhD[1], Cagnie B. PhD[1]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium
[2] Pain in Motion international research group, www.paininmotion.be
[3] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium
[4] School of Psychology, Faculty of Health Sciences, Australian Catholic University, Australia

*equally contributed first authors

Pain Physician, 2017; 20(4): 245-260.
SCI_{2015}= 3.40, 5-year impact factor= 3.694, Q2 in anesthesiology (8/31), Q2 in clinical neurology (53/193)

Abstract

Background: Chronic neck pain affects 50-85% of people who have experienced an acute episode. This transition and the persistence of chronic complaints is believed to be mediated by brain alterations among different central mechanisms.

Objectives: This study aimed to systematically review and critically appraise the current existing evidence regarding structural and functional brain alterations in patients with whiplash associated disorders (WAD) and idiopathic neck pain (INP). Additionally, associations between brain alterations and clinical symptoms reported in neck pain patients were evaluated.

Setting: This systematic review examined brain imaging studies available on PubMed and/or Web of Science that analyzed structural and functional brain alterations in patients with WAD and INP.

Methods: The present systematic review was performed according to the PRISMA guidelines. PubMed, Web of Science, and Cochrane databases were searched. First, the obtained articles were screened based on title and abstract. Secondly, the screening was based on full-text. Risk of bias in included studies was investigated.

Results: Twelve studies met the inclusion criteria. Alterations in brain morphology and function, including perfusion, neurotransmission and blood oxygenation level dependent-signal, were demonstrated in chronic neck pain patients. There is some to moderate evidence for both structural and functional brain alterations in patients with chronic neck pain. In contrast, no evidence for structural brain alterations in acute neck pain patients was found.

Limitations: Only 12 articles were included, which imposes the possibility to draw only cautious conclusions.

Conclusion: Brain alterations were observed in both patients with chronic WAD and chronic INP. Furthermore, more evidence exists for brain alterations in chronic WAD, and different underlying mechanisms might be present in both pathologies. Furthermore, pain and disability were correlated with the observed brain alterations. Accordingly, morphological and functional brain alterations should be further investigated in patients with chronic WAD and chronic INP with newer and more sensitive techniques, and associative clinical measurements seem indispensable in future research.

Key words: traumatic neck pain, idiopathic non-traumatic neck pain, brain alterations, Magnetic Resonance Imaging, Single Photon Emission Computed Tomography, Photon Emission Tomography, chronic neck pain.

Introduction

Chronic neck pain affects many people who have encountered an episode of neck pain throughout their lifetime with 50-85% of people who experienced neck pain reporting neck pain 1 – 5 years later [1, 2].

Two groups of non-specific neck pain patients have been identified in the literature, i.e. patients who have developed neck pain after a traumatic event, which are referred to as patients with whiplash associated disorders (WAD) [3], and patients with non-traumatic idiopathic neck pain (INP). Whiplash injuries usually result from rear-end motor vehicle collisions resulting in acceleration-deceleration mechanisms of forces acting on the neck and the head [4]. The traumatic neck pain group is frequently seen as a special case as these patients develop more often chronic complaints [5, 6], which do not only consist of neck pain and/or headaches, but also include other symptoms such as dizziness [7], motor dysfunction [8-13], disturbed central pain processing or central sensitization [14, 15], and cognitive impairment [5, 16-19], hence the term "associated disorders". In addition, patients with INP have developed neck pain without any clear underlying cause [20]. These patients are mostly not characterized by central sensitization [21], cognitive impairment and dizziness, however they also display motor dysfunctions [22, 23], and chronic or recurrent pain.

The cause of this diversity in symptoms observed in patients suffering from acute and chronic pain is still not entirely clear. Some have suggested that alteration of the central nervous system could explain this diversity [24-28], and a theoretical framework for central nervous system alterations, such as brain alterations, has already been constructed for acute and chronic pain [29, 30]. Surprisingly, only few have tried to analyze and publish results that might support these theories. In addition, it is known that a trauma can result in mild traumatic brain injury (MTBI) [31], which is associated with clinical symptoms similar to these observed in patients with WAD. However, information on the impact of a whiplash trauma on the brain remains scarce in patient with neck pain. To answer the question if brain alterations play a role in patients suffering from chronic pain, the application of new brain analysis tools is rising [32-37]. However, only limited research is available on alterations in brain morphology and function in patients with WAD and INP.

Brain alterations are often categorized into functional alterations, and morphological or structural alterations. Brain function, which reflects the amount of "activity" that the brain generates at a certain location, is often measured via its blood perfusion and/or metabolism. Single Photon Emission Computed Tomography (SPECT) and Photon Emission Tomography (PET) are both applied for this purpose [37]. Both methods use radio-pharmaceutical tracers to assess the brain's perfusion and/or metabolism. Active brain regions have a higher need of oxygen and glucose, which is reflected in a higher perfusion

and/or metabolism [38, 39]. These changes in metabolite concentration are captured and reflected in the image signal intensity. Another method to analyze brain function is by applying functional Magnetic Resonance Imaging (MRI). The MRI method most often used to provide information related to brain function is called blood oxygenation level dependent (BOLD) contrast imaging [40]. This method is based on MR-images made sensitive to changes in oxygen consumption with an increase in consumption reflecting higher signal intensities in these BOLD-images.

Besides brain function, brain morphology is also believed to be altered in certain pain conditions [41, 42]. MRI has achieved the level of golden standard for measuring brain morphology, typically through voxel- or surface-based methods [43]. Both methods are able to provide information on white and grey matter volume.

According to our knowledge, no systematic review has critically summarized the current evidence regarding brain alterations in patients with WAD, and patients with INP. This systematic review is able to determine the present state of the art and steer further research in patients with WAD and INP. The aim of this systematic review is to review and critically appraise the current existing evidence related to structural and functional brain alterations in patients with WAD and INP. In addition, this review evaluates the association between these brain alterations and the different clinical symptoms reported in patients with neck pain.

Methods

Protocol

This systematic review applies the guidelines issued in the PRISMA statement, an adaptation of the QUORUM statement for reporting systematic reviews [44, 45].

Information sources

The electronic databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Cochrane Library (http://www.cochranelibrary.com), and Web of Science (http://www.webofscience.com) were searched to identify relevant articles. Additionally, to make the search as complete as possible, reference lists of the eligible papers were screened. Databases were searched on the 4th of December 2015, and all articles were screened afterwards on eligibility criteria.

Literature search strategy

The search strategy was based on a combination of Mesh-terms (only for searching PubMed) and free text words derived from the following PICO format: participants (P) had to suffer from acute, subacute or chronic INP, neck injuries or WAD; the measurement instrument (I) had to include medical brain imaging techniques such as MRI, PET, and SPECT; and the outcome (O) had to refer to brain alterations including brain function, and brain morphology. The complete entered search strategy in PubMed was ("neck pain" OR "Neck Pain"[Mesh] OR "whiplash" OR "Whiplash Injuries"[Mesh] OR "neck injury" OR "Neck Injuries"[Mesh]) AND ("brain imaging" OR "Neuroimaging"[Mesh] OR "fMRI" OR "rs-fMRI" OR "MRI" OR "Magnetic Resonance Imaging"[Mesh] OR "Magnetic Resonance Imaging" OR "Positron Emission Topography" OR "PET" OR "Positron-Emission Tomography"[Mesh] OR "CT" OR "Computed Tomography" OR "SPECT" OR "Tomography, X-Ray Computed"[Mesh]) AND ("brain morphology" OR "Brain"[Mesh] OR "brain" OR "white matter" OR "grey matter" OR "gray matter" OR "White Matter"[Mesh] OR "Gray Matter"[Mesh] OR "brain function" OR "resting state" OR "BOLD" OR " brain volume").

Eligibility criteria

Only patient-control, cross-sectional and cohort studies reporting on brain alterations in non-specific non-traumatic and traumatic acute, subacute or chronic neck pain patients were eligible for inclusion in this systematic review. No restrictions on publication date or status were imposed. Studies had to be written in English, Dutch or French to be included in this systematic review. Adult participants of age older than 18 suffering from INP or a whiplash injury were considered eligible. Assessment of brain alterations should focus on brain structure/morphology and/or brain function, and only imaging techniques, such as SPECT, PET, and MRI were considered to be included in this systematic literature review.

Study selection

Two reviewers, BC and RDP, independently screened all articles on eligibility in a standardized manner. Disagreement between the reviewers was resolved by consensus.

Data collection process and items

Data were extracted from eligible papers in a standardized manner by RDP, and the extracted data were checked afterwards by a second reviewer, IC, who made changes where necessary. Disagreements were resolved by consensus or the opinion of a third reviewer, MM. Extracted data consisted of: author and year of publication, description of the included population and if available the controls (sample size, neck pain type, mean age, sex, duration of complaints before scanning), the imaging protocol (SPECT, PET or MRI) with technical information on the scanning sequence or radiopharmaceutical tracers used in the scanning procedure, the brain tissue class and areas that were investigated during the scanning sequence, and the main findings and associations with clinical measures (Spearman correlations (r_s), Pearson correlations (r_p)).

Risk of bias in individual studies

To assess the methodological quality of all eligible papers, two independent reviewers, RDP and IC, both PhD candidates experienced in conducting systematic reviews, screened all articles on risk of bias using a modified version of "The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analyses" (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) [46]. This checklist is recommended for case-control and cohort studies [47] and has been proposed by the Cochrane Collaboration (www.cochrane.org). Two different checklists were used to assess the quality of cross-sectional case-control and cohort studies. The case-control checklist evaluates selection of the population (case definition, representativeness of cases and selection of controls) and comparability (controlled for the most important confounders such as age, sex, education level, and BMI). The cohort checklist evaluates selection (representativeness of the cohort, selection controls, case definition, new cases), comparability (controlled for the most important confounders (age, sex, education level, BMI)), and exposure (follow-up). In addition, for both checklists, four scoring items, specifically developed for the content of this systematic review, were added (description of MRI protocol, quality control of images, blindness of researcher, same method used for cases and controls). Further details on the different criteria are displayed in **table 2.** Each cross-sectional study could reach a maximum score of 9 and each cohort study could reach a maximum score of 11 on the modified NOS, representing the highest methodological quality.

Based on the study design and risk of bias, a level of evidence was given to every study, according to the 2005 classification system of the Dutch Institute for Healthcare Improvement CBO (**supplementary table 1**). Prospective cohort trials of sufficient size and follow-up that have adequately controlled for confounding, and selective follow-up is sufficiently excluded, obtained a level of evidence A2, while cohort studies not meeting these criteria or case-control studies obtained a level of evidence B. Non-controlled trials and expert opinions obtained a level of evidence C and D, respectively.

Strength of conclusion

Subsequently, the strength of conclusion (ranging from 1 to 4) was calculated for each cluster of studies reflecting one outcome parameter (**supplementary table 2**), and was placed between brackets in the results section. Strength of conclusion 1 was assigned for a study of level A1 or at least two independently conducted studies of level A2. Strength of conclusion 2 was given when at least two independently conducted studies of evidence level B or one trial of evidence level A2 was included in the cluster, and strength of conclusion 3 was assigned if one study of evidence level B or C was present. Strength of conclusion 4 was given in case of inconclusive or inconsistent results between various studies.

Results

Study selection

In total, 477 studies were retrieved from the different databases. After the first screening, 26 studies were identified as potential eligible studies for inclusion. After the second screening, only 12 studies were retrieved that fulfilled all inclusion-criteria. The selection process of relevant articles is presented in **figure 1**.

Study characteristics

The characteristics of each study were extracted and presented in the evidence table (**Table 1**). Four studies reported on structural brain alterations in neck pain patients [48-51], using a different MRI protocol. One study reported on changes in BOLD-signal through task-related fMRI-imaging [52], another one reported on alterations in neurotransmission through PET imaging [53], and six studies analyzed perfusion and/or metabolism via PET or SPECT-imaging using radiopharmaceutical tracers [54-59]. The average age (+/- SD) of the total patient and control sample, when available, was 36.41 (+/-11.52) years and 33.0 (+/-12.21) years respectively. In total, 75% (n=179) of the study participants were female. Eleven studies reported on patients after a whiplash injury or patients with WAD. Both longitudinal [49, 50] and cross-sectional studies [48, 51, 52, 54-59] have been included, using time periods ranging from 14 days to more than 1 year after the whiplash trauma before performing the scanning protocol. The key-findings of this systematic review are also depicted in **figures 2** and **3**.

Risk of bias in individual studies

Cross-sectional studies obtained a score ranging from 2/9 (22%) to 8/9 (89%) for risk of bias with a median score of 4.5/9 (50%). The risk of bias in studies was mostly high due to a lack of representativeness of cases in 6 out of 10 studies (60%) [55-59], indicating the lack of a random sample. In six cases (60%), the researchers were not blinded for the patient's condition [53-57, 59], and six authors (60%) did not clearly describe how the quality of the images was assessed [48, 51, 54, 56, 57, 59]. However, only one study (10%) did not provide a qualitative description of the cases included in their studies [57], and one study (10%) did not provide a detailed brain imaging protocol [48]. Cohort-studies, of which only two were eligible for this systematic review [49, 50], obtained a score of 3/11 (27%) and 6/11 (54%). Both studies lost points on the representativeness of cases, selection of controls, new cases, quality control of images and blinding the researchers for the patient's condition. In most cases (96%), the 2 reviewers (RDP & IC) agreed. After a second review and a comparison of the differences, the reviewers reached a consensus for all items. The risk of bias in the individual studies is presented in **Table 2**.

Table 1 Evidence table

Author (year)	Patient population	Controls	Imaging protocol	Brain measure	Main Findings	Level of Evidence
Alterations in morphology						
Borchgrevinck et al. (1997)[48]	n = 46; **whiplash-injury within 2 days** (acute); no WAD* IV; 26 ♀ and 20 ♂; age 35,7y +/- 11,5	n=20; healthy controls; employees hospital; 34,5y +/- 10,2	Sagittal T1-spin echo; Transversal and Coronal T2-turbo spin echo	**structural data:** Lesions, CSF, cerebral sulci	NS	**B**
Karlsborg et al. (1997)[49]	**Whiplash-injury within 14 days** (acute) **and after 7 months** (chronic): n = 39; 23 ♀; 33y (WAD*-II, III and IV)	*NA*	T2-spin echo sequence	**structural:** edema	NS	**C**
Obermann et al. (2009)[50]	**Post-traumatic headache within 14 days**: (acute) n = 32; 35,2y +/- 12,1; 20 ♀	n = 30; students or members hospital; 17 ♀; 35y +/- 13,7	T1-weighted image	**structural data:** VBM (grey matter volume changes)	NS	**A2**
	after 3 months and 1 year (chronic): n = 12; 39,6y +/- 15,1; 7 ♀	non-chronic posttraumatic headaches (n = 20)			**3 months**: significant decrease in ACC and DLPFC; **1 year**: resolved; **increase:** brainstem (PAG), thalamus and cerebellum.	
Sturzenegger et al. (2008)[51]	**WAD*-I or WAD*-II:** n = 21; > 5 months (chronic); 71% ♀	n = 18; 72% ♀	T1-MPRAGE	**VBR**: Ventricle-Brain Ratio's (Diffuse Axonal Injury)	**VBR**: WAD = CON	**B**
Alterations in function						
Alterations in BOLD-signal						
Freitag et al. (2001)[52]	**WAD* II:** n = 5; 43,2y; **WAD* 0:** n = 5; 33,2y; between 14 and 34 months (chronic)	n = 7; 35,0y	T2*-fMRI (1,5T Siemens)	**BOLD-response**: middle temporal and middle superior temporal	**BOLD:** WADII < WAD0; WADII < CON (middle temporal and middle superior temporal)	**B**
Alterations in neurotransmission						
Linmann et al. (2010)	**WAD*-II**: > 6 months (chronic); n = 18; 14 ♀; 38y +/- 11	n = 18; 9 ♀; 35y +/- 9	^{11}C (NK1 specific radioligand) ^{15}O-PET	**Neurotransmission:** NK1-receptor availability (pain processing)	**NK1-receptor availability**: WAD < Controls: R. Ventromedial Prefrontal Cortex, Insula, R. Middle cingulate cortex, L. Hippocampus, L. Amygdala, PAG NK1-receptor availability correlates negatively with TSK, however not with pain duration or disability (NDI) WAD: vmPFC NK1-receptor availability correlates negatively with rCBF in R. subgenual ACC NK1-receptor availability alterations: independent of between group rCBF differences	**B**
Alterations in perfusion/metabolism						
Bakhtadze et al. (2012)[54]	**Chronic neck pain** (> 3 months) n = 45; 40y +/- 10,9; 16 ♂ and 29 ♀	*NA*	SPECT (HMPAO)	**Perfusion**: frontal, temporal, parietal, occipital (both hemispheres)	**Perfusion**: moderate and severe neck pain < mild (parietal and frontal); NDI and VAS scores correlates with SPECT-score.	**C**

Table 1 Continued

Author (year)	Patient population	Controls	Imaging protocol	Brain measure	Main Findings	Level of Evidence
Alterations in perfusion/metabolism						
Linnman et al. (2009)[55]	**WAD*-II patients**: n = 21; 17 ♀; 37y +/- 11; pain between 6 and 24 months (chronic)	n = 18; 9 ♀; 35y +/- 9	^{15}O-PET	**rCBF**: different brain regions	rCBF: **WAD < CON:** Middle temporal gyrus, Middle occipital gyrus; **WAD > CON**: Left posterior corpus calossum; Left parahippocampal gyrus; posterior cingulate gyrus; lingual gyrus; Right posterior corpus calossum; Right parahippocampal gyrus, posterior cingulate gyrus, caudate nucleus; Right inferior temporal gyrus; Right Thalamus; Right Cerebellum, anterior lobe, dentae; Right middle frontal gyrus, Left precentral gyrus, right postcentral gyrus. rCBF right middle frontal gyrus ~ NDI (r_p = 0.52, NS) rCBF right temporo-occipital zone ~ NDI (r_p = -0.53, NS) rCBF right precentral gyrus ~ Pain rating (r_p = 0.52, NS)	**B**
Sundström et al. (2006)[56]	**Whiplash:** n = 27; 41y; > 3 years (chronic); 18 ♀	**INP:** n = 18; 44y; > 3 years; 13 ♀	SPECT (HMPAO)	**rCBF**	rCBF: **INP < CON:** Right Temporal Lobe, Right temporal gyrus, Left cerebellum culmen, parahippocampal regions; **WAD < CON:** NS; **INP < WAD:** Right temporal lobe; **WAD < INP:** NS	**B**
Otte et al. (1997)[57]	**Whiplash-syndrome:** n = 6; 41 +/- 17y; between 3 and 63 months (chronic)	n = 12; 44 +/- 19y	FDG(18F)-PET; ECD-SPECT	**Perfusion and metabolism** pariëto-occipital	**Perfusion & metabolism**: whiplash < control (parieto-occipital)	**B**
Lorberboym et al. (2002)[58]	**Post-whiplash**: n = 20; > 6 months (chronic)	*NA*	SPECT (HMPAO)	**Perfusion**	13 patients: **perfusion** abnormalities; 8 in temporal lobes; 3 in occipital lobes; 2 in frontal lobes; 2 in basal ganglia	**C**
Radanov et al. (1999)[59]	**Whiplash-injury patients:** n = 21; 11 ♀; 42y +/- 8,6; between 6 and 48 months (chronic)	*NA*	SPECT (HMPAO); ^{15}O-PET; 18F-PET	**Perfusion**	NS	**C**

Abbreviations: n, amount of participants; y, age in years; ♀, women; ♂, men; CON, controls; WAD, whiplash associated disorders; INP, idiopathic neck pain; NS, not significant; <, smaller then; > larger then; NK1, Neurokinin 1; TSK, Tampa Scale for Kinesiophobia; NDI, Neck Disability Index; L., Left; R., Right; r_p, Pearson correlation coefficient; NS, Not Significant; NA, not applicable; rCBF, regional cerebral blood flow; CSF, cerebrospinal fluid; SPECT, Single Photon Emission Computed Tomography; PET, Photon Emission Tomography; PAG, periaqueductal grey; ACC, anterior cingulate cortex; HMPAO, hexamethylpropyleneamine oxime; vmPFC, ventromedial prefrontal cortex; *WAD-classification according to Spitzer et al. (1995)[3].

Synthesis of results

Alterations in morphology

Two studies have examined structural abnormalities shortly after the occurrence of a whiplash injury (within 2 to 14 days), but found no signs of edema in the acute whiplash group [48] nor signs of lesions when they compared patients after a whiplash injury with healthy controls [49]. Another study analyzed the ventricle-brain ratio (VBR) - calculated as the ratio of the total ventricle volume divided by the brain volume and normalized afterwards by accounting for the average distance between the frontal and occipital poles of the entire study group [51]. The authors found no difference in VBR between patients with chronic WAD and healthy controls [51]. A study that performed voxel-based morphometry in patients with post-traumatic headache and neck pain, found no structural brain alterations in the acute phase [50]. However, decreased grey matter volume in the Anterior Cingulate Cortex (ACC) and Dorsolateral Prefrontal Cortex (DLPFC) was observed after 3 months. These grey matter changes did, however, resolve after one year coinciding with the cessation of the post-traumatic headache. In comparison, an increase in grey matter volume was observed in the periaqueductal grey matter (PAG), the thalamus and cerebellum [50].

To conclude, moderate evidence exists for a lack of structural pathological brain abnormalities, such as edema or lesions in acute traumatic neck pain patients who have suffered from a whiplash injury (Strength of conclusion 2). Moderate evidence exists for grey matter volume alterations after a certain period in time in patients with post-traumatic headache (Strength of conclusion 2).

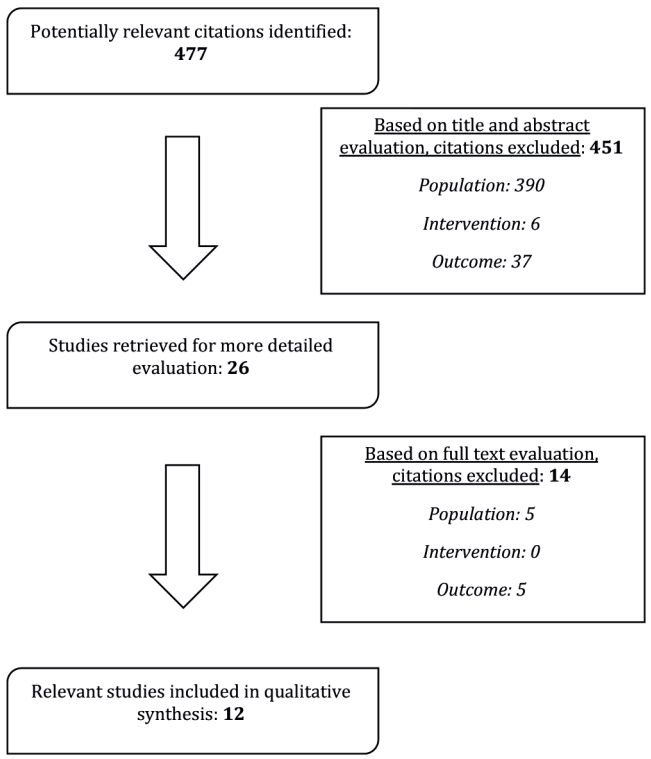


Figure 1 Study selection process.

Alterations in function (BOLD-signal)

One study examined the BOLD-signal at visual areas (middle temporal and superior temporal) in patients with chronic WAD [52]. Therefore, patients were exposed to a visual task in the scanner. Two fields of dots were presented to the patient by projection. One field was positioned in the left visual field, whereas the other field was presented in the right visual field. On both screens dots moved in random directions, and the subjects had to fixate their eyes on a central spot. At random coherent movement of dots was added to one of both screened resulting in a mixture of random and coherent motion, and the screen with this mixture had to be reported by the patients to the researchers. The authors demonstrated a lower BOLD-response during coherent motion perception in the symptomatic chronic WAD group compared to asymptomatic persons after a whiplash trauma and healthy controls.

In conclusion, some evidence exists for functional brain alterations in temporal regions in patients with chronic whiplash (Strength of conclusion 3).

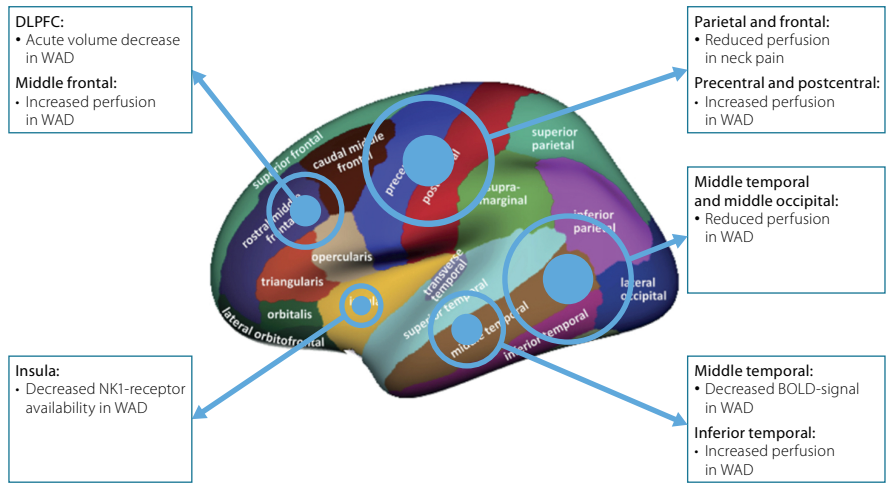


Figure 2 Lateral view of the brain with key-findings regarding brain alterations.

Figure from "An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest, Desikan et al., (2006). NeuroImage, 31(3):968-80."

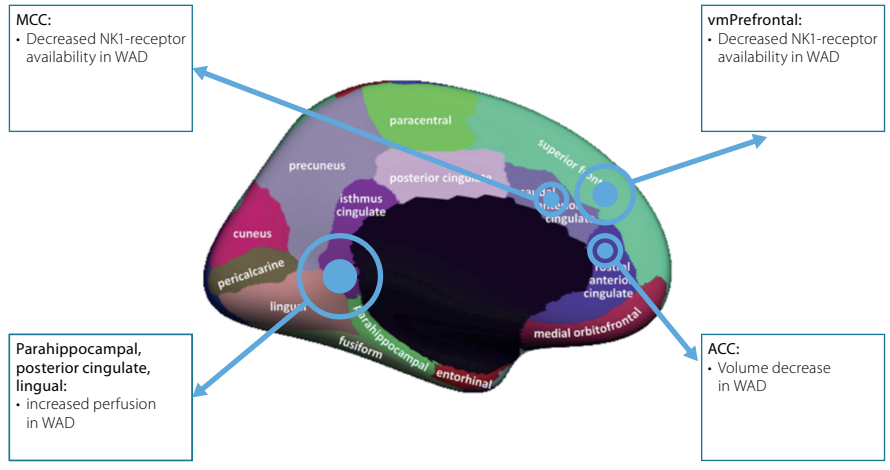


Figure 3 Medial view of the brain with key-findings regarding brain alterations.

Figure from "An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest, Desikan et al., (2006). NeuroImage, 31(3):968-80."

Table 2 Methodological quality of included studies

Cross-sectional study design

	Case definition[1]	Representativeness[2]	Selection Controls[3]	Comparability[4]	MRI[5a]	Quality control[5b]	Blindness[5c]	Same method[6]	Total /9 (%)
Borchgrevink et al. (1997)	+	+	+	+	-	-	+	+	**6 (67)**
Sundström et al. (2006)	+	-	++	+	+	-	-	-	**5 (55)**
Linnman et al. (2009)	+	-	-	+	+	+	-	+	**5 (55)**
Sturzenegger et al. (2008)	+	+	++	+	+	-	+	+	**8 (89)**
Radanov et al. (1999)	+	-	-	-	+	-	-	+	**3 (25)**
Otte et al. (1997)	-	-	-	-	+	-	-	+	**2 (22)**
Lorberboym et al. (2002)	+	-	-	-	+	+	+	-	**4 (44)**
Linnman et al. (2010)	+	+	++	+	+	+	-	+	**8 (89)**
Freitag et al. (2001)	+	-	+	-	+	+	+	+	**6 (67)**
Bakhtadze et al. (2012)	+	+	-	-	+	-	-	-	**3 (25)**

+ = score fulfilled; - = score not fulfilled
Modified Newcastle-Ottawa Quality Assessment Scale: cross-sectional studies: **1=**Is the case definition adequate? (Independent validation or self-reported); **2**=Representativeness of cases (Random sample: description of area, hospital and clinic); **3**=Selection of controls (Community controls with no history of disease (++), Hospital controls with no history of disease (+)); **4**=Comparability (Controlled for the most important confounders (age, sex, cognition, BMI)); **5a**=Description of MRI protocol (full description and optimal sequencing protocol); **5b**=Quality control of images (fully descripted); **5c**=Blindness (researchers was blinded for patient's status); **5d**=Same method used for controls/cases (yes).

Cohort study design

	Representativeness[1]	Selection Controls[2]	Ascertainment of exposure[3]	New cases[4]	Comparability[5]	MRI[6a]	Quality control[6b]	Blindness[6c]	Same method[7]	Follow-up[8]	Total /11 (%)
Karlsborg et al. (1997)	-	-	+	-	-	-	-	-	-	++	**3 (27)**
Obermann et al. (2009)	-	-	+	-	+	+	-	-	+	++	**6 (54)**

+ = score fulfilled; - = score not fulfilled
1=Representativeness of exposed cohort (truly representative average in the community); **2**=Selection controls (Drawn from the same community); **3**=Ascertainment of exposure (Independent validation or self-reported); **4**=New cases (Yes); **5**=Comparability (Controlled for the most important confounders (age, sex, cognition, BMI)); **6a**=Description of MRI protocol (Full description and optimal sequencing protocol); **6b**=Quality control of images (fully descripted); **6c**=Blindness (researcher was blinded for patient's status); **7**=Same method used for controls/cases (yes); **8**=Follow-up (Long enough (>3months) and > 80% (++), >80% (+)).

Alterations in function (neurotransmission)

Only one study investigated functional brain alterations related to neurotransmission [53]. Linnman et al. (2010) analyzed the Neurokinin 1 (NK1)-receptors, a receptor mostly mediated by the neuropeptide Substance P (SP), which allows the regulation of affective behavior and nociception [60]. In their study a decrease of NK1-receptor availability was found in chronic WAD patients [53], which was observed in the insula, right middle cingulate cortex, left hippocampus, left amygdala, and the PAG, but most distinct in the right ventromedial prefrontal cortex (vmPFC). Furthermore, these changes were negatively correlated with the scores on the self-reported Tampa Scale for Kinesiophobia (TSK) Questionnaire. Also, vmPFC NK1-receptor availability was negatively correlated with regional cerebral blood flow (rCBF) in the right subgenual ACC in chronic WAD patients.

In conclusion, some evidence exists for a decrease in NK1-receptor availability in pain processing brain regions in patients with chronic WAD (Strength of conclusion 3). Furthermore, there is some evidence *that decreased vmPFC NK1-receptor availability is negatively correlated with rCBF in the ACC in chronic WAD patients (Strength of conclusion 3).*

Alterations in perfusion/metabolism

In total, six studies examined alterations in perfusion/metabolism of the brain through SPECT or PET-imaging [54-59]. Only one study found no indication for changes of brain perfusion [59]. Sundström et al (2006) demonstrated that patients with chronic INP showed a decreased rCBF pattern compared to healthy controls, which was most obvious in the parahippocampal and temporal regions, and the cerebellum. In contrast, no such alterations could be observed in patients suffering from chronic WAD compared with healthy controls [61]. Linnman et al (2009) analyzed the rCBF in patients with chronic WAD and found alterations in the left parahippocampal gyrus, lingual gyrus, and posterior cingulate gyrus [55]. In addition, alterations were also evident in the right parrahippocampal gyrus, caudate nucleus, pulvinar nucleus of the thalamus, and posterior cingulate gyrus. Perfusion appeared higher in these regions in patients with chronic WAD, whereas in regions around the temporo-occipital transition zone, a decreased perfusion was found in these patients [55]. In a more recent study of Bakhtadze et al. (2012), a decreased perfusion of the parietal and frontal region was found in patients who suffered from moderate to severe chronic INP symptoms when compared to patients with only mild symptoms. Moreover, perfusion (SPECT-score) correlated with the amount of pain-related disability (utilizing the Neck Disability Index) [54]. Two studies analyzed the perfusion in chronic WAD after a period of 6 months, and both found abnormalities in the patient group [56, 58]. One study found predominantly decreased perfusion rates in patients with chronic INP compared to healthy controls in the right temporal gyrus, and left cerebellum culmen [56]. However, no changes were found in the observation of perfusion in chronic WAD [56]. This is in contrast with the study of Lorberboym et al. (2002), who found perfusion abnormalities in 13 of the 20 included patients with chronic WAD. However, these abnormalities were not equal for all patients. In 8 patients, perfusion abnormalities were observed in the temporal lobes, in 3 patients in the occipital lobes, in 2 patients in the frontal lobes, and another 2 patients showed perfusion abnormalities in the basal ganglia [58].

To conclude, moderate evidence exists for alterations in brain perfusion and metabolism in chronic neck pain patients (Strength of conclusion 2), however the nature and location of these alterations is not entirely obvious. In addition, two studies found contradictory results for the association between clinical parameters and brain perfusion/metabolism in patients with chronic neck pain, resulting in strength of conclusion 4.

Discussion

This systematic review aimed to summarize and critically appraise the current state of the literature concerning brain alterations in patients with WAD and INP. Researchers have attempted to construct a solid hypothesis regarding the appearance of brain alterations in chronic pain and its associated symptoms [62, 63]. However, only some to moderate evidence exists for empirical findings of structural and functional brain alterations in patients with chronic neck pain.

Some of the included studies scored poorly on the modified NOS, signifying the potential risk of bias included in the sample of studies discussed in this review. It is important that in future research a random sample of the study population, and the healthy controls is included. New neuroimaging research indicates an important role of confounders in brain research, such as sex [64], age [65-69], BMI [70, 71], medication use [72, 73] and cognitive function [74]. These confounders should be included by future neuroimaging studies into their statistical analysis to avoid biased estimates. About 75% of the included population were females, which is similar with current epidemiological data of (chronic) neck pain patients [1]. In addition, most studies reported no statistical significant difference in age between the patient population and the included controls.

Morphological brain alterations

Although evidence for structural brain alterations in patients with chronic neck pain remains scarce, we can conclude that no structural brain abnormalities seem to be present in the acute phase after a whiplash trauma. Different authors observed nor signs of edema or lesions [48, 49], nor underlying structural differences between patients with chronic posttraumatic headache, acute posttraumatic headache and healthy controls within 14 days after the accident, which makes morphometric predisposition in the affected patient group less likely [50]. However, more longitudinal studies with a sufficiently large follow-up period are necessary to confirm these results and to determine a time frame for possible changes. After a three-month period, decreased grey matter volume in the ACC and

DLPFC was observed in whiplash patients who have developed chronic headache. Yet, these changes were observed to resolve after one year in concurrence with the cessation of the headache. In contrast, an increased grey matter volume was observed in the PAG, thalamus and cerebellum after one year in these patients with chronic headache [50]. These regions are known to be involved in pain sensation. The ACC and DLPFC are involved in the salience and affective-cognitive dimension of pain, and may play an important role in pain modulation, exerting top down inhibition [75-77]. A disruption of the grey matter integrity of these regions might alter pain sensation. One study did identify the DLPFC as a site of major neurodegeneration in chronic pain patients [78], potentially leading to an increased pain sensation [79]. In addition, increased grey matter volume of the PAG was demonstrated in patients with chronic WAD [50]. The PAG is a brainstem structure that is part of the descending pain modulatory network and is crucially involved in pain inhibition or antinociception. These changes are furthermore in accordance with the current literature, where authors found primarily an increase of grey matter morphology in the brain stem [75]. It has been suggested that the observed neuroplasticity (increase and decrease of grey matter morphology) might result from an aim to restore the balance between nociceptive and antinociceptive modulation [50]. These changes in morphology seem to be very different across different pathologies and depend on timing [41]. This diversity might correspond with the diversity of perfusion abnormalities in patients with chronic WAD found by Lorberboym et al. (2002), which are discussed in the section on functional brain alterations [58].

Sturzenegger et al. (2008) found no difference in VBR in patients suffering from chronic WAD, indicating their sample did not show any signs of diffuse axonal injury (DAI). They neither observed a correlation between VBR and any neuropsychological test [51]. These results are somewhat surprising as diffuse axonal injuries have already been observed in patients with MTBI [80], who suffer from similar symptoms as patients with WAD. Also, Caeyenberghs et al. (2010) have reported the presence of an association between postural control and visuomotor tracking, and a measure of DAI in patients with TBI [81, 82]. The use of Pearson correlations could contribute to the reason for not finding any associations between DAI and the patient's symptoms, as this measure only analyzes a linear relationship between two variables. Furthermore, the authors stated that the method they used to evaluate brain tissue loss could not be sensitive enough to detect very mild diffuse focal atrophy [51]. A newer and potentially more sensitive technique for assessing white matter tracts is diffusion weighted imaging (DWI), a technique that indirectly analyses the coherence of motion of protons [83, 84].

Functional brain alterations

One study examined alterations in neurotransmission by looking at the density of NK1–receptors in the brain [53], which are widely distributed throughout the brain with high density in the striatum, the amygdala and the DLPFC [85]. This receptor allows the

modulation of pain via the neuropeptide SP, known to be elevated in patients with chronic WAD [86]. Both, SP and NK1, have been implicated in locomotive activity [87] and in pain processing [88]. Therefore, it is not surprising that the forebrain not only acts as a "top-down" pain inhibitor, but also modulates voluntary movement by altering the inhibition exerted by the basal ganglia on motor output, resulting in pain induced immobilization [53]. A decrease in NK1-receptor availability, which was most pronounced in the vmPFC of patients with chronic WAD [53] may provide forebrain modulation through its dense projections to the striatum - globus pallidus complex [89], which is supported by the observed negative correlation between kinesiophobia and NK1-receptor availability [55]. Consequently, high endogenous levels of SP could result in attenuation of NK1 function in the vmPFC, starting a negative vicious circle of increased avoidance [55]. Furthermore, decreased grey matter volume in the forebrain (vmPFC) in pain processing regions was already observed in patients with chronic complex regional pain syndrome together with reduced white matter integrity [90].

Alterations in the brain perfusion of patients with chronic INP and chronic WAD have been observed by different studies [54-59]. The diversity of results in brain perfusion and metabolism might be attributed to both methodological differences and technical issues. The use of different tracers results in different observations. Oxygen-15 (15-O) is for example often applied to determine blood flow, while fluorine-18 (18-F), which is injected as glucose, provides information on the brain's metabolism. Some authors state that blood flow provides more accurate information on brain function compared to glucose consumption, as it is more sensitive to neural activation [91]. These differences make comparison between both methods rather difficult. The comparison between SPECT and PET imaging remains challenging due to differences in technical features, such as the use of different tracers and sensitivity, with PET being more sensitive to neural events [37]. Thus, only cautious interpretations can be drawn from the comparison between studies that applied different imaging methods. The diversity in brain perfusion alterations within one technique could again support the hypothesis of individual adaptations to restore the equilibrium between nociceptive and antinociceptive modulation [58]. Surprisingly, many brain areas were found to exhibit a higher amount of rCBF, which may reflect a compensation mechanism for regional brain atrophy [92]. In addition, one study found differences between patients with chronic INP and patients with WAD [56]. These results might suggest that different mechanisms underlie the transition to a chronic or recurrent pain state in both patients group, which is in accordance with other study areas [28].

Although the direction of brain perfusion alterations is still unclear, most authors do agree on the presence of perfusion alterations in patients with chronic neck pain. Furthermore, research suggests the presence of an association between the patient's self-reported disability (NDI and pain ratings) and cerebral perfusion [54, 55]. Therefore, future research should rather focus on the association between brain alterations and the severity

of self-reported disability [54], although differences in brain activation between patients with chronic WAD and chronic idiopathic neck pain were already observed [61]. Only one study did not find any associations between brain activation and neuropsychological tests of divided attention and working memory [59].

Limitations and strengths

When interpreting the results, the following study limitations have to be taken into consideration. Firstly, only 12 articles were included in this review, which imposes the possibility to draw only cautious conclusions. Secondly, the diversity in brain imaging techniques hampers the possibility of comparison, as we are aware of the different technical features in every technique, which potentially affect the observed outcome. Thirdly, authors tend to use different "brain atlases" for analyzing and describing their results. Some analyzed a global region, such as the temporal lobe, while others investigated specific parts of a certain lobe, such as the vmPFC. Many researchers have tried to address a certain function to the brain's anatomy and have attributed a specific function to certain brain areas [93, 94]. Lastly, many studies did suffer from certain risks of bias, which could affect their results, and this implies the impossibility to draw firm conclusions from the current literature.

However, also several strengths of this systematic review can be outlined. Firstly, the present systematic review is innovative and is valuable to steer future brain research. Secondly, the methods used for screening and scoring were completed by two independent blinded researchers. At last, the NOS checklist was modified by adding 2 MRI-related scoring items specifically developed for the topic of the current systematic review. Consequently, the methodological quality of the MRI articles could be evaluated more thoroughly giving a more accurate view on the MRI data acquisition, processing and quality control.

Recommendations for further research

Future studies should certainly try to avoid bias, and should consider the mentioned considerations regarding the inclusion of confounding factors. Furthermore, more longitudinal research could allow exploring the causal relationship between brain imaging results and the development and maintenance of persistent neck pain. In addition, research on disease-specific neck pain could reveal different neuroplastic brain changes when compared to non-specific neck pain.

To date, the imaging techniques used in studies that have assessed the brain in a population of neck pain patients are outdated. Recently, new morphological brain analysis tools were developed with new features allowing a more detailed assessment of the human brain. Surface-based morphometry (SBM) has yielded better results in terms of specificity compared to voxel-based morphometry [32, 43]. One reason is the ability of SBM to assess not only volumetric measurements, but also measures of cortical thickness and

cortical surface area. Alterations in thickness and area cause different clinical effects, and non-uniformity of these changes has been shown in the brain in aging [95]. DWI has also yielded better results in terms of analyzing the microstructural organization of white matter bundles [83]. Besides improvement in morphological imaging, functional brain imaging has also evolved. One new popular method, defined as "resting state fMRI" could give more insight into the functional organization of the brain during rest [96]. Recently, a new theoretical framework has risen which addresses the influence of alterations in the aggregation of different functional components of the brain and its influence on pain [4]. Lastly, alterations in the network of the brain have been given attention in different conditions, including chronic pain conditions [97]. Assessing the morphological and functional brain network could allow to assess the macroscopic organization of this complex organ [98].

Conclusion

Some to moderate evidence exists for both structural and/or functional brain alterations in patients with chronic INP or WAD. In the acute phase, no structural alterations were found, but if symptoms persisted, changes in different brain areas were demonstrated. Although, most authors agree that brain alterations are present in both patients with chronic WAD and patients with chronic INP, there is currently more evidence for brain alterations in chronic whiplash patients, and different underlying mechanisms might be present in both pathologies. Moreover, brain alterations observed in chronic neck pain patients are very diverse, indicating multiple mechanisms are responsible for the brain's neuroplasticity associated with the presence of pain. Pain and disability seems to be furthermore correlated with the observed brain alterations. Based on our results, morphological and functional brain alterations should be further investigated in patients with chronic WAD and chronic INP via more sophisticated and sensitive techniques.

References

1. Hogg-Johnson S, van der Velde G, Carroll LJ, Holm LW, Cassidy JD, Guzman J, Cote P, Haldeman S, Ammendolia C, Carragee E, Hurwitz E, Nordin M, Peloso P. The burden and determinants of neck pain in the general population - Results of the bone and joint decade 2000-2010 task force on neck pain and its associated disorders. *Eur Spine J* 2008:17: S39-S51.
2. Carroll LJ, Hogg-Johnson S, van der Velde G, Haldeman S, Holm LW, Carragee EJ, Hurwitz EL, Côté P, Nordin M, Peloso PM, Guzman J, Cassidy JD. Course and Prognostic Factors for Neck Pain in the General Population: Results of the Bone and Joint Decade 2000 –2010 Task Force on Neck Pain and Its Associated Disorders. *Eur Spine J* 2008:17: 75-82.
3. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine* 1995:20: 1s-73s.
4. Davis CG. Mechanisms of chronic pain from whiplash injury. *J Forensic Leg Med* 2013:20: 74-85.
5. Sterling M, Jull G, Kenardy J. Physical and psychological factors maintain long-term predictive capacity post-whiplash injury. *Pain* 2006:122: 102-108.
6. Rebbeck T, Sindhusake D, Cameron I, Rubin G, Feyer A, Walsh J, Gold M, Schofield W. A prospective cohort study of health outcomes following whiplash associated disorders in an Australian population. *Inj Prev* 2006:12: 93-98.
7. Treleaven J. Sensorimotor disturbances in neck disorders affecting postural stability, head and eye movement control. *Man Ther* 2008:13: 2-11.
8. Baydal-Bertomeu JM, Page ÁF, Belda-Lois JM, Garrido-Jaén D, Prat JM. Neck motion patterns in whiplash-associated disorders: quantifying variability and spontaneity of movement. *Clinical Biomechanics* 2011:26: 29-34.
9. Dall'Alba PT, Sterling MM, Treleaven JM, Edwards SL, Jull GA. Cervical range of motion discriminates between asymptomatic persons and those with whiplash. *Spine* 2001:26: 2090-2094.
10. Grip H, Sundelin G, Gerdle B, Karlsson JS. Variations in the axis of motion during head repositioning–A comparison of subjects with whiplash-associated disorders or non-specific neck pain and healthy controls. *Clin Biomech* 2007:22: 865-873.
11. Sjölander P, Michaelson P, Jaric S, Djupsjöbacka M. Sensorimotor disturbances in chronic neck pain—range of motion, peak velocity, smoothness of movement, and repositioning acuity. *Man Ther* 2008:13: 122-131.
12. Woodhouse A, Stavdahl Ø, Vasseljen O. Irregular head movement patterns in whiplash patients during a trajectory task. *Exp brain res* 2010:201: 261-270.
13. Meisingset I, Woodhouse A, Stensdotter A-K, Stavdahl Ø, Lorås H, Gismervik S, Andresen H, Austreim K, Vasseljen O. Evidence for a general stiffening motor control pattern in neck pain: a cross sectional study. *BMC musculoskelet disord* 2015:16: 56.
14. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013:17: 299-312.
15. Stone AM, Vicenzino B, Lim EC, Sterling M. Measures of central hyperexcitability in chronic whiplash associated disorder--a systematic review and meta-analysis. *Man Ther* 2013:18: 111-117.
16. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain physician* 2015:18: E389-401.
17. Treleaven J, Jull G, Sterling M. Dizziness and unsteadiness following whiplash injury: characteristic features and relationship with cervical joint position error. *J Rehabil Med* 2003:35: 36-43.
18. Rubin AM, Woolley SM, Dailey VM, Goebel JA. Postural stability following mild head or whiplash injuries. *Otol Neurotol* 1995:16: 216-221.
19. Kessels RP, Aleman A, Verhagen WI, Van Luijtelaar EL. Cognitive functioning after whiplash injury: a meta-analysis. *J Int Neuropsychol Soc* 2000:6: 271-278.
20. Borghouts JA, Koes BW, Bouter LM. The clinical course and prognostic factors of non-specific neck pain: a systematic review. *Pain* 1998:77: 1-13.
21. Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. *Clin J Pain* 2005:21: 175-181.
22. De Pauw R, Coppieters I, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? - A systematic review. *Man Ther* 2015.
23. Woodhouse A, Vasseljen O. Altered motor control patterns in whiplash and chronic neck pain. *BMC Musculoskelet Disord* 2008:9: 90.
24. Hodges PW, Tucker K. Moving differently in pain: a new theory to explain the adaptation to pain. *Pain* 2011:152: S90-98.
25. Heikkilä HV, Wenngren B-I. Cervicocephalic kinesthetic sensibility, active range of cervical motion, and oculomotor function in patients with whiplash injury. *Arch Phys Med Rehabil* 1998:79: 1089-1094.
26. Wenngren BI, Pettersson K, Löwenhielm G, Hildingsson C. Eye motility and auditory brainstem response dysfunction after whiplash injury. *Acta Otolaryngol* 2002:122: 276-283.
27. Mosimann UP, Müri RM, Felblinger J, Radanov BP. Saccadic eye movement disturbances in whiplash patients with persistent complaints. *Brain* 2000:123: 828-835.
28. De Pauw R, Coppieters I, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? - A systematic review. *Man Ther* 2015.
29. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 2005:9: 463-484.
30. Apkarian AVBMNGPY. Towards a theory of chronic pain. *Prog Neurobiol* 2009:87: 81-97.
31. Alexander MP. Mild traumatic brain injury: pathophysiology, natural history, and clinical management. *Neurol*1995.
32. Fischl B. FreeSurfer. *Neuroimage* 2012:62: 774-781.
33. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000:11: 805-821.
34. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. *Neurotherapeutics* 2007:4: 316-329.
35. Tournier JD, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magn Reson Med* 2011:65: 1532-1556.
36. Jeurissen B, Leemans A, Tournier JD, Jones DK, Sijbers J. Investigating the prevalence of complex fiber configurations in white matter tissue with diffusion magnetic resonance imaging. *Hum Brain Mapp* 2013:34: 2747-2766.
37. Rahmim A, Zaidi H. PET versus SPECT: strengths, limitations and challenges. *Nucl Med Commun* 2008:29: 193-207.
38. Warwick JM. Imaging of brain function using SPECT. *Metab Brain Dis* 2004:19: 113-123.
39. Dienel GA, Hertz L. Glucose and lactate metabolism during brain activation. *J Neurosci Res* 2001:66: 824-838.
40. Amaro E, Barker GJ. Study design in fMRI: basic principles. *Brain Cogn* 2006:60: 220-232.
41. Baliki MN, Schnitzer TJ, Bauer WR, Apkarian aV. Brain Morphological Signatures for Chronic Pain. *PLoS ONE* 2011:6: e26010-e26010.
42. Schmidt-Wilcke T, Ganssbauer S, Neuner T, Bogdahn U, May A. Subtle grey matter changes between migraine patients and healthy controls. *Cephalalgia* 2008:28: 1-4.
43. Clarkson MJ, Cardoso MJ, Ridgway GR, Modat M, Leung KK, Rohrer JD, Fox NC, Ourselin S. A comparison of voxel and surface based cortical thickness estimation methods. *Neuroimage* 2011:57: 856-865.
44. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010:8: 336-341.
45. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015:4: 1.
46. Wells G SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. 2014. Available from: http://www.ohri.ca/programs/clinical_epidemiology/ oxford.asp.
47. Zeng X, Zhang Y, Kwong JS, Zhang C, Li S, Sun F, Niu Y, Du L. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. *J Evid Based Med* 2015:8: 2-10.
48. Borchgrevink G, Smevik O, Haave I, Haraldseth O, Nordby A, Lereim I. MRI of cerebrum and cervical columna within two days after whiplash neck sprain injury. *Injury* 1997:28: 331-335.

49. Karlsborg M, Smed A, Jespersen HF, Stephensen SL, Cortsen ME, Jennum PJ, Herning GM, Korfitsen E, Werdelin LM. [Whiplash injury syndrome. A prospective study of 39 patients with whiplash injury]. *Ugeskr Laeger* 1998:160: 6211-6215.
50. Obermann M, Nebel K, Schumann C, Holle D, Gizewski ER, Maschke M, Goadsby PJ, Diener HC, Katsarava Z. Gray matter changes related to chronic posttraumatic headache. *Neurol* 2009:73: 978-983.
51. Sturzenegger M, Radanov BP, Winter P, Simko M, Farra AD, Di Stefano G. MRI-based brain volumetry in chronic whiplash patients: no evidence for traumatic brain injury. *Acta Neurol Scand* 2008:117: 49-54.
52. Freitag P, Greenlee MW, Wachter K, Ettlin TM, Radue EW. fMRI response during visual motion stimulation in patients with late whiplash syndrome. *Neurorehabil Neural Repair* 2001:15: 31-37.
53. Linnman C, Appel L, Furmark T, Soderlund A, Gordh T, Langstrom B, Fredrikson M. Ventromedial prefrontal neurokinin 1 receptor availability is reduced in chronic pain. *Pain* 2010:149: 64-70.
54. Bakhtadze MA, Vernon H, Karalkin AV, Pasha SP, Tomashevskiy IO, Soave D. Cerebral perfusion in patients with chronic neck and upper back pain: preliminary observations. *J Manipulative Physiol Ther* 2012:35: 76-85.
55. Linnman C, Appel L, Soderlund A, Frans O, Engler H, Furmark T, Gordh T, Langstrom B, Fredrikson M. Chronic whiplash symptoms are related to altered regional cerebral blood flow in the resting state. *Eur J Pain* 2009:13: 65-70.
56. Sundstrom T, Guez M, Hildingsson C, Toolanen G, Nyberg L, Riklund K. Altered cerebral blood flow in chronic neck pain patients but not in whiplash patients: a 99mTc-HMPAO rCBF study. *Eur Spine J* 2006:15: 1189-1195.
57. Otte A, Mueller-Brand J, Nitzsche EU, Wachter K, Ettlin TM. Functional brain imaging in 200 patients after whiplash injury. *J Nucl Med* 1997:38: 1002.
58. Lorberboym M, Gilad R, Gorin V, Sadeh M, Lampl Y. Late whiplash syndrome: correlation of brain SPECT with neuropsychological tests and P300 event-related potential. *J Trauma* 2002:52: 521-526.
59. Radanov BP, Bicik I, Dvorak J, Antinnes J, von Schulthess GK, Buck A. Relation between neuropsychological and neuroimaging findings in patients with late whiplash syndrome. J Neurol Neurosurg Psychiatry 1999:66: 485-489.
60. Mantyh PW. Neurobiology of substance P and the NK1 receptor. *J Clin Psychiatry* 2001:63: 6-10.
61. Sundstrom T, Guez M, Hildingsson C, Toolanen G, Nyberg L, Riklund K. Altered cerebral blood flow in chronic neck pain patients but not in whiplash patients: a Tc-99m-HMPAO rCBF study. *Eur Spine J* 2006:15: 1189-1195.
62. Schmidt-Wilcke T. Neuroimaging of chronic pain. *Best Pract Res Clin Rheumatol* 2015:29: 29-41.
63. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 2015:87: 474-491.
64. Passe TJ, Rajagopalan P, Tupler LA, Byrum CE, Macfall JR, Krishnan KRR. Age and sex effects on brain morphology. *Prog Neuropsychopharmacol Biol Psychiatry* 1997:21: 1231-1237.
65. Lemaitre H, Goldman AL, Sambataro F, Verchinski Ba, Meyer-Lindenberg A, Weinberger DR, Mattay VS. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol Aging* 2012:33: 617.e611-617.e619.
66. Grady CL, Springer MV, Hongwanishkul D, McIntosh AR, Winocur G. Age-related changes in brain activity across the adult lifespan. *J Cogn Neurosci* 2006:18: 227-241.
67. Meunier D, Achard S, Morcom A, Bullmore E. Age-related changes in modular organization of human brain functional networks. *Neuroimage* 2009:44: 715-723.
68. Burke SN, Barnes CA. Neural plasticity in the ageing brain. *Nat Rev Neurosci* 2006:7: 30-40.
69. Ceko M, Bushnell MC, Fitzcharles MA, Schweinhardt P. Fibromyalgia interacts with age to change the brain. *Neuroimage Clin* 2013:3: 249-260.
70. Pannacciulli N, Del Parigi A, Chen K, Le DSN, Reiman EM, Tataranni PA. Brain abnormalities in human obesity: a voxel-based morphometric study. *Neuroimage* 2006:31: 1419-1425.
71. Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM. Brain structure and obesity. *Hum brain mapp* 2010:31: 353-364.
72. Walther K, Bendlin B, Glisky E, Trouard T, Lisse J, Posever J, Ryan L. Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. *Neurobiol Aging* 2011:32: 497-505.
73. Younger JW, Chu LF, D'Arcy NT, Trott KE, Jastrzab LE, Mackey SC. Prescription opioid analgesics rapidly change the human brain. *Pain* 2011:152: 1803-1810.
74. Bendlin BB, Fitzgerald ME, Ries ML, Xu G, Kastman EK, Thiel BW, Rowley HA, Lazar M, Alexander AL, Johnson SC. White matter in aging and cognition: a cross-sectional study of microstructure in adults aged eighteen to eighty-three. *Dev Neuropsychol* 2010:35: 257-277.
75. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. *J Neuroimmune Pharmacol* 2013:8: 518-534.
76. Baliki MN, Apkarian AV. Nociception, pain, negative moods, and behavior selection. *Neuron*, 2015:87(3), 474-491.. 2015.
77. Vachon-Presseau, E., Centeno, M. V., Ren, W., Berger, S. E., Tétreault, P., Ghantous, M., ... & Apkarian, A. V. The Emotional Brain as a Predictor and Amplifier of Chronic Pain. *Dent Res J* 2016.
78. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci* 2004:24: 10410-10415.
79. Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. *Brain* 2003:126: 1079-1091.
80. Inglese M, Makani S, Johnson G, Cohen BA, Silver JA, Gonen O, Grossman RI. Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. *J Neurosurg* 2005:103: 298-303.
81. Caeyenberghs K, Leemans A, Geurts M, Taymans T, Linden CV, Smits-Engelsman B, Sunaert S, Swinnen SP. Brain-behavior relationships in young traumatic brain injury patients: DTI metrics are highly correlated with postural control. *Hum brain mapp* 2010:31: 992-1002.
82. Caeyenberghs K, Leemans A, Geurts M, Taymans T, Vander Linden C, Smits-Engelsman B, Sunaert S, Swinnen SP. Brain-behavior relationships in young traumatic brain injury patients: fractional anisotropy measures are highly correlated with dynamic visuomotor tracking performance. *Neuropsychologia* 2010:48: 1472-1482.
83. Jones DK, Knösche TR, Turner R. White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. *Neuroimage* 2013:73: 239-254.
84. Tournier J-D, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magn Reson Med* 2011:65: 1532-1556.
85. Hietala J, Nyman MJ, Eskola O, Laakso A, Grönroos T, Oikonen V, Bergman J, Haaparanta M, Forsback S, Marjamäki P. Visualization and quantification of neurokinin-1 (NK1) receptors in the human brain. *Mol Imaging Biol* 2005:7: 262-272.
86. Alpar EK, Onuoha G, Killampalli VV, Waters R. Management of chronic pain in whiplash injury. *Journal of bone and joint surgery-british volume-* 2002:84: 807-811.
87. Elliott PJ, Iversen SD. Behavioural effects of tachykinins and related peptides. *Brain Res* 1986:381: 68-76.
88. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000:288: 1765-1768.
89. Haber S, Kunishio K, Mizobuchi M, Lynd-Balta E. The orbital and medial prefrontal circuit through the primate basal ganglia. *J Neurosci* 1995:15: 4851-4867.
90. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron* 2008:60: 570-581.
91. Varvatsoulias G. The physiological processes underpinning PET and fMRI techniques with an emphasis on the temporal and spatial resolution of these methods. *Psychological Thought* 2013:6: 173-195.
92. Dickerson BC, Salat DH, Bates JF, Atiya M, Killiany RJ, Greve DN, Dale AM, Stern CE, Blacker D, Albert MS. Medial temporal lobe function and structure in mild cognitive impairment. *Ann Neurol* 2004:56: 27-35.
93. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 2006:129: 564-583.
94. Alvarez JA, Emory E. Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol Rev* 2006:16: 17-42.
95. Lemaitre H, Goldman AL, Sambataro F, Verchinski BA, Meyer-Lindenberg A, Weinberger DR, Mattay VS. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol Aging* 2012:33: 617. e611-617. e619.
96. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: A review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* 2010:20: 519-534.
97. Crossley NA, Mechelli A, Scott J, Carletti F, Fox PT, McGuire P, Bullmore ET. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. *Brain* 2014:137: 2382-2395.
98. Rubinov M, Sporns O. Complex network measures of brain connectivity: Uses and interpretations. *Neuroimage* 2010:52: 1059-1069.

Supplementary Table 1 Level of evidence, according to the 2005 classification system of the Dutch Institute for Healthcare Improvement CBO

	Intervention
A1	Systematic review of at least 2 independent from each other conducted studies of evidence level A2
A2	Randomized double-blinded comparative clinical research of good quality and efficient size
B	Comparative research, but not with al characteristics as mentioned for A2. This includes also patient-control research and cohort research.
C	Not comparative research
D	Opinion of experts

www.cbo.nl

Supplementary Table 2 Strength of Conclusion (modified table)

	Conclusion based on
1	Research of evidence level A1 or at least 2 independent conducted studies of evidence level A2
2	1 research of evidence level A2 or at least 2 independent conducted studies of evidence level B
3	1 research of evidence level B or C
4	Inconclusive or inconsistent results between various studies

PART II

Differences between traumatic and idiopathic chronic neck pain: interrelationships among disability, cognitive deficits, central sensitization, and structural brain alterations

Original research papers

Chapter 3

Differences between women with traumatic and idiopathic chronic neck pain and women without neck pain: interrelationships among disability, cognitive deficits, and central sensitization

Coppieters I. MSc[1, 2]; De Pauw R. MSc[1]; Kregel J. MSc[1, 2, 4]; Malfliet A. MSc[1, 2, 4]; Goubert D. MSc[1]; Lenoir D. MSc[1]; Cagnie B. PhD[1,*]; Meeus M. PhD[1, 2, 3,*]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium
[2] Pain in Motion international research group, www.paininmotion.be
[3] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium
[4] Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Faculty of Physical Education & Physiotherapy, Free University Brussels, Belgium

*equally contributed last authors

Physical Therapy, 2017; 97:338-353.
SCI_{2015}= 2.799, 5-year impact factor= 3.680, Q1 in Orthopaedics (10/74), Q1 & D1 in Rehabilitation (5/65))

Abstract

Background: To date, a clear differentiation of disability, cognitive deficits, and central sensitization between chronic neck pain of traumatic nature and that of a nontraumatic nature is lacking.
Objective: This study aimed to examine differences in disability, cognitive deficits, and central sensitization between women with traumatic and idiopathic (nontraumatic) chronic neck pain and women who were healthy. In addition, interrelationships among these variables were investigated.
Design: This was a case-control study.
Methods: Ninety-five women (28 women who were healthy (controls), 35 women with chronic idiopathic neck pain (CINP), and 32 women with chronic whiplash-associated disorders (CWAD) (traumatic) were enrolled in the study. First, all participants completed standardized questionnaires to investigate pain-related disability and health-related quality of life. Next, cognitive performance was assessed. Finally, pressure pain thresholds and conditioned pain modulation were examined to investigate central sensitization.
Results: Pain-related disability, reduced health-related quality of life, and cognitive deficits were present in participants with CWAD and, to a significant lesser extent, in participants with CINP ($p<.017$). Local hyperalgesia was demonstrated in participants with CWAD ($p<.001$) and CINP ($p<.017$) but not in women who were healthy. However, distant hyperalgesia and decreased conditioned pain modulation efficacy were shown only in participants with CWAD ($p<.017$); this result is indicative of the presence of central sensitization. Moderate to strong Spearman correlations ($\rho = .456 – .701$) among disability, cognitive deficits, and hyperalgesia (local and distant) were observed in participants with CWAD ($p<.01$). In participants with CINP, only local hyperalgesia and subjective cognitive deficits were moderately ($\rho = .463$) correlated ($p<.01$).
Limitations: No conclusions about the causality of the observed correlations can be drawn.
Conclusions: This innovative research revealed important differences between women with CWAD and women with CINP and thus provided evidence of the clinical importance of distinguishing the assessment and rehabilitation approaches for both pain conditions.

Key words: chronic whiplash associated disorders, chronic idiopathic neck pain, central sensitization, disability, quality of life, cognitive performance

Introduction

Chronic neck pain is one of the most prevalent musculoskeletal pain disorders worldwide [1] and is associated with high socioeconomic and personal health costs, and extensive psychosocial burden, and frequent medical physician and physical therapist consultations [2-4]. Moreover, the prevalence is still increasing; therefore, the socioeconomic burden is growing [5].

Chronic neck pain can be subdivided, on the basis of its etiology, into 3 main categories: specific neck pain, trauma-induced neck pain, and idiopathic (nontraumatic) neck pain. This article focuses on chronic neck pain of a traumatic or an idiopathic nature.

Chronic idiopathic neck pain (CINP) is defined as neck pain lasting more than 3 months, without the presence of specific causes, such as trauma, cervical hernias with clinical symptoms, or radiculopathy. Often no underlying structural pathology can be demonstrated [6, 7] and radiological imaging findings are poorly related to a patient's clinical symptoms [6, 8]. Scientific research concerning the factors contributing to pain persistence in CINP is limited.

Chronic traumatic neck pain is characterized by persistent neck pain lasting more than 3 months and resulting predominantly from a whiplash injury [9]. Whiplash injuries usually originate from rear-end motor vehicle collisions and are caused by acceleration-deceleration forces acting on the neck [10]. The trauma and indirect impact at the cervical spine may lead to the development of various clinical manifestations defined as *chronic whiplash-associated disorders* (CWAD) [11, 12]. Apart from persistent pain, the main other complaints reported by patients with CWAD are concentration difficulties, psychosocial problems, fatigue, headache, and reduced quality of life [12-15].

Research regarding the underlying pathophysiology of CWAD is extensive. There is compelling evidence for the presence of various cervical dysfunctions, such as impaired cervical movement control [16-18] and increased cervical muscle tone [19], and there is some evidence for alterations in neck muscle morphology [20]. However, the contributions of these dysfunctions to the complex clinical scenario for patients with CWAD seem rather limited [21].

Besides local neck problems, there is compelling evidence for the presence of central sensitization in patients with CWAD [13, 22-24]. Central sensitization is defined as "an increased responsiveness of central nociceptive-signaling neurons to normal or subthreshold afferent input" [25]. Distant hyperalgesia, enhanced bottom-up nociceptive transmission [13], and inefficient activation of endogenous pain inhibition [26] have been demonstrated in

patients with CWAD [27, 28]. However, the underlying mechanisms of central sensitization are still largely unclear, and the relationship between central sensitization, and other clinical symptoms in patients with CWAD compared to patients with CINP has been poorly investigated.

Besides persisting pain, patients with CWAD commonly have pain-related disability and reduced quality of life [13, 29-31]. Depression and disability, which have a negative impact on quality of life, are associated with poor recovery in patients with CWAD [29].

Other important complaints in these patients are decreased cognitive capabilities, which have been demonstrated with cognitive tests [13, 32]. Soon after the initial injury, patients with whiplash report difficulties with concentration and memory, and these complaints continue into the chronic phase [33]. However, the association between cognitive deficits and the development and maintenance of chronic pain is unclear.

Most research has focused solely on CWAD and has not addressed CINP. Scientific research into differences between the underlying mechanisms of chronic neck pain of traumatic origin (CWAD) and those of chronic neck pain of nontraumatic origin (CINP) is highly limited. On the basis of the scarce literature comparing CWAD and CINP, indications for different underlying mechanisms can be found [34]. First, one study of central sensitization indicated that sensory hypoesthesia, altough present in CWAD, is not a feature of CINP [35]. Second, a recent systematic review concluded that central sensitization and sensory abnormalities outside the cervical spine are rare in patients with CINP [36]. However, the results of these few studies were inconclusive. Finally, biomedical studies focusing on dysfunctions in cervical muscle morphology and performance comparing CWAD and CINP also revealed differences [16, 17, 37].

Accordingly, these findings indicate that CWAD and CINP are separate clinical conditions [34], and it can be hypothesized that the traumatic origin of chronic neck pain in patients with CWAD gives rise to deficits more severe than those seen in patients with CINP. Nevertheless, experimental studies exploring differences in disability and cognitive performance between both chronic neck pain conditions are essentially lacking. Moreover, associations among disability, cognitive deficits, and central sensitization have been hypothesized but remain largely unexplored. Gaining more insight into possible differences between and interrelationships among these variables could contribute to more effective assessment and therapy approaches for both pain conditions. Differentiation in management between CINP and CWAD may be necessary to improve clinical outcomes.

For the reasons outlined above, the first aim of this study was to examine differences in disability, cognitive deficits, and central sensitization between people with traumatic chronic neck pain (CWAD) and CINP and people who were healthy. More deficits in these variables were presumed to be present in people with CWAD than in those with CINP. The second aim was to investigate significant relationships among measures of disability, cognitive deficits, and central sensitization in both chronic neck pain conditions.

Methods

Study design and setting

This cross-sectional case-control study took place in the research laboratories of the Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Ghent, Belgium. The study was performed from February 2014 to September 2015. The research protocol was approved by the local Ethics Committee of the Ghent University Hospital (EC/2013/1053) and was in compliance with the Declaration of Helsinki.

Participants

Ninety-five women (35 women with CINP, 32 women with CWAD, and 28 women who were healthy and pain-free (controls)) were enrolled in the study. Only women were included because research has demonstrated significant differences in pain sensitivity, pressure pain thresholds (PPTs), and efficacy of endogenous pain inhibition between men and women [38-40]. All participants were Dutch native speakers and 18 to 65 years old. Participants were recruited by calls on social media and through advertisements placed in health magazines and an information brochure of an association for patients with whiplash, on the Ghent University website, and via local radio. Furthermore, informative flyers and posters were distributed in different medical institutes and associations in Flanders, Belgium (various hospitals, physical therapist practices, and medical physician practices).

Inclusion criteria for people with traumatic chronic neck pain and CINP were persistent neck pain lasting more than 3 months, with a mean pain intensity of more than 3 of 10 on a numeric pain rating scale during the preceding month, which is the cut-off for clinically relevant pain [41]. All participants with chronic neck pain had to report mild/moderate to severe pain-related disability, established by a score of 10 or more of a maximum of 50 on the Neck Disability Index (NDI) [42]. Furthermore, participants with chronic neck pain had to report stability of pain medication intake for at least 4 weeks before study participation.

People with CWAD were included only if they had neck pain resulting from a motor vehicle crash or traumatic event and classifiable as WAD II A, B, or C on the modified [12] Quebec Task Force Scale [43].

The inclusion criterion for people with CINP was persistent idiopathic (nontraumatic) neck pain. People with CINP were excluded if they ever experienced a whiplash trauma. In addition, people with specific causes of neck pain, such as cervical hernias with clinical symptoms, were excluded.

Women who were healthy and pain-free could participate only if they were pain-free on the test day (numeric pain rating scale score of <2/10), had no history of neck-shoulder-arm pain for more than 8 consecutive days during the preceding year, with a pain intensity of 2 or more on the numeric pain rating scale; no medical consultation for neck-shoulder-arm pain during the preceding year; and no history of whiplash trauma. Additionally, women who were healthy and pain-free were included only if they had a score of less than 8 on the NDI.

General exclusion criteria for all study groups were the presence of major depression or psychiatric illness; neurologic, metabolic, cardiovascular disorders; inflammatory conditions; fibromyalgia; chronic fatigue syndrome; and a history of neck or shoulder girdle surgery. Furthermore, women who were pregnant and women who were 1 year postnatal were excluded to preclude confounding factors. All participants were asked to discontinue intake of nonopioid analgesics 48 hours before study participation. In addition, participants were asked to avoid heavy physical exertion and to refrain from consuming alcohol, caffeine, and nicotine on the day of testing. All participants were thoroughly informed about the study procedures and signed an informed consent statement before study enrolment.

Procedure

First, all participants completed a general survey to acquire information on demographics and education level. Subsequently, disability measures were obtained with 2 validated Dutch questionnaires. All participants completed these questionnaires online and in a fixed order. On the test day, participants reported neck pain duration and scored current neck pain intensity on a numeric pain rating scale (0-10). Afterward, assessments to investigate cognitive performance and central sensitization were performed. All tests were carried out by the same experienced researcher.

Outcome measures

Disability

Neck pain-related disability

The NDI was used to investigate neck pain-related disability levels (0-50) [42, 44]. Higher scores on the NDI indicate higher levels of pain-related disability. The Dutch language version of the NDI has been proven to be reliable and valid for patients with chronic neck pain [45, 46].

Health-related quality of life

For the evaluation of health-related quality of life, the Medical Outcomes Study 36-Item Short-Form Health Survey Questionnaire (SF-36) was used [47]. This self-report questionnaire can be divided into 2 main domains of health: the physical and mental health components. The SF-36 total score (0-800) is the summation of the physical health (0-500) and mental health (0-300) component scores. Higher scores represent better health for that particular health component. The Dutch language version of the SF-36 has been shown to have good reliability and validity in adults who are healthy and patients with chronic pain [48].

Cognitive performance

Self-perceived cognitive performance

For the evaluation of self-reported cognitive deficits, participants completed the Dutch modified Perceived Deficits Questionnaire (mPDQ) (0-72). This questionnaire investigates self-perceived cognitive symptoms in 4 different cognitive subdomains (prospective memory, retrospective memory, attention and concentration, and organization and planning) during the preceding 4 weeks; symptoms are rated on a 5-point Likert scale anchored from never (0) to almost always (4). Higher scores represent more self-perceived cognitive symptoms. The mPDQ is the modified version of the Perceived Deficits Questionnaire, which was adapted for patients with CWAD [49]. The validity and reliability of the English language version of the mPDQ have been demonstrated in patients with CWAD and people who are healthy [49].

Objective cognitive performance

The Trail Making Test (TMT) was administered to objectively obtain an instrumented measure of cognitive performance [50]. First, in part A, the participants were asked to connect 25 numbers in ascending order as fast as possible. Second, in part B, the participants had to alternate between numbers and letters. The goal of the TMT was to finish part A and part B as quickly as possible while still maintaining accuracy. The time taken to complete each part of the test was used as an outcome measure. Higher scores represent more deficits.

In addition, the ratio of scores on TMT part B to scores on TMT part A and the difference between scores on TMT part B and scores on TMT part A were calculated. These scores can elucidate the added task requirements of TMT part B and are purer indexes of the complex cognitive processes involved in part B. The difference between TMT part B and TMT part A removes the speed component from the TMT assessment and minimizes visuoperceptual and working memory requirements, thus providing an indication of executive function [50]. Furthermore, the ratio of TMT part B to TMT part A diminishes the influence of psychomotor demands and controls on intrasubject variability factors, thus focusing on the measurement of cognitive flexibility [51].

Central sensitization

Self-perceived symptoms of central sensitization

All participants completed the Dutch language version of the Central Sensitization Inventory (CSI). The CSI is a self-report screening instrument for the measurement of clinical symptoms of central sensitization (0-100) in people with chronic pain [52, 53]. The Dutch CSI has been shown to have good internal consistency, good discriminative power, and excellent test-retest reliability [52]. Neblett et al [54] determined that a CSI score of 40 of 100 best distinguished between a group of patients who had a central sensitivity syndrome (CSI scores of ≥ 40/100) and a group of patients who did not have a central sensitivity syndrome (area under the curve = .86, sensitivity = 81%, specificity = 75%). The CSI is a valid instrument for screening patients for the possible presence of central sensitization, although the chances of false-positive results are relatively high when patients with complex pain disorders are evaluated [55].

Experimental measures of central sensitization

For investigation of the presence of central sensitization, 2 critical aspects of central pain processing were assessed with experimental pain tests: PPTs and conditioned pain modulation (CPM).

Pressure pain hyperalgesia

Pressure pain thresholds were measured unilaterally with a digital pressure algometer (FDX™, Wagner Instruments, Greenwich, Connecticut), both at a symptomatic local region (middle trapezius muscle midway between the spinous process of C7 and the lateral border of the acromion) to evaluate local hyperalgesia and at asymptomatic distant regions (quadriceps muscle midway between the anterior superior iliac spine and the basis patellae, the skin web between the thumb and the index finger, and the lumbar level 5 cm lateral to the spinous process of L3) to evaluate distant hyperalgesia [56, 57] (Fig. 1). Pressure pain thresholds were assessed on the more painful side [58]. In women who were healthy and when participants with CINP and CWAD experienced the same amount of neck pain on both sides, PPTs were tested on the dominant side.

Pressure pain thresholds were assessed in a randomized order (with Research Randomizer; https://www.randomizer.org). During the test procedure, participants were comfortably seated and pressure was gradually increased at a rate of 1 kgf/s until the participants reported the first sensation of unpleasantness. The PPT was determined as the mean of 2 consecutive (30 seconds in between) measurements. This technique was found to be reliable [59]. In addition, the intratester reliability of PPT measurements is satisfactory to good (intraclass correlation coefficient= .78 - .93) [60].

Conditioned Pain Modulation

The presence of dysfunctional endogenous pain inhibition was investigated by evaluating the efficacy of CPM by applying a CPM paradigm. This paradigm relies on the "pain-inhibits-pain" mechanism, in which a noxious stimulus is used as a conditioning stimulus to induce a reduction in the perception of pain from another test stimulus [61, 62]. The conditioning stimulus for eliciting CPM was the cold pressor test. The assessment of PPTs was used as the test stimulus.

First, participants were given clear instructions about the test procedure. Next, one hand was immersed in water maintained at room temperature (22°C) for 1 minute to standardize the hand temperature, as previously described by other researchers [63]. Subsequently, participants were asked to immerse the same hand (up to the wrist) in a bath (VersaCool, Thermo Fisher Scientific, Waltham, Massachusetts) with circulating cold water maintained at 12°C (SD=1°C) [64]. The contralateral hand to the test stimulus (PPT measurement) was used to maximize the CPM effect, which is dependent on the distance between the stimuli [65]. Participants were asked to keep their hand in the water bath for 2 minutes [63]. During the conditioning stimulus, after 25, 60, and 114 seconds, participants rated the perceived pain intensity of the cold water on an 11-point verbal numeric rating scale with responses ranging from 0 ("no pain") to 10 ("worst imaginable pain").

Pressure pain thresholds were re-evaluated at 2 of the predefined locations (trapezius muscle and quadriceps muscle) in the same randomized order as before. The PPT re-evaluation started at 30 seconds after immersion of the hand (Fig. 1). If participants removed the hand from the water before the end of the 2 minutes, the measurement was registered as missing. For analyses of CPM efficacy, the mean PPT measured before the cold pressor test was subtracted from the mean PPT measured during the cold pressor test. Hence, a lower CPM value reflected less efficient endogenous pain inhibition. Intrasession and intraclass correlation coefficients for the cold pressor test have been shown to be excellent (.85) [66].

Data analysis

All statistical analyses were performed with IBM SPSS Statistics 22.0 (IBM SPSS, Armonk, New York). First, the normality of variables was checked with the Shapiro-Wilk test and by visual evaluation of quantile-quantile plots and histograms. Additionally, the equality of variance was examined with the Levene's test. Only normally distributed data with an equality of variance were analysed with parametric tests. Otherwise, non-parametric tests were applied.

The comparability of study groups for age, current neck pain intensity, neck pain duration, and other demographics was explored with a 1-way analysis of variance (post hoc Bonferroni), the Kruskal-Wallis test (post-hoc Mann-Whitney *U* test), or Fisher exact test. Subsequently, differences in disability, cognitive deficits, and central sensitization between participants with CWAD and CINP and women who were healthy were explored

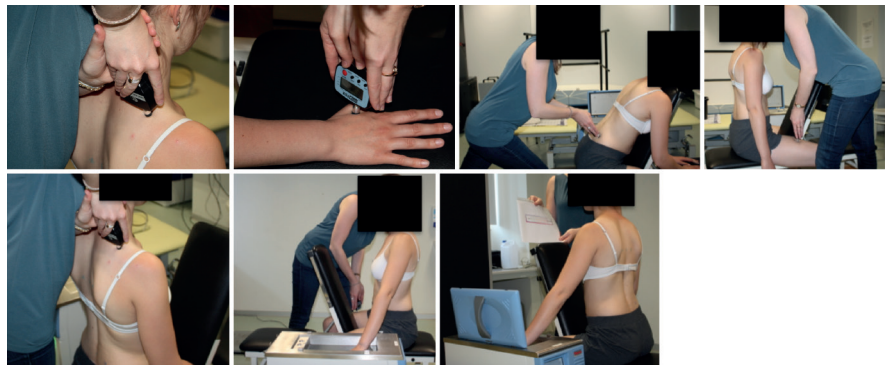


Figure 1 Top row: the assessment of local and distant hyperalgesia by measuring pressure pain thresholds at the trapezius muscle (local hyperalgesia); the web between thumb and index finger (= *hand*), lumbar region (= *low back*), and quadriceps muscle (distant hyperalgesia). Bottom row: the assessment of conditioned pain modulation efficacy at the trapezius muscle, and at the quadriceps muscle.

with a 1-way analysis of variance (post hoc Bonferroni) or the Kruskal-Wallis test (post hoc Mann-Whitney *U* test).

Finally, correlations among measures of disability, cognitive deficits, and central sensitization in both chronic neck pain conditions were investigated with Spearman correlation analyses.

To correct for multiple comparisons, we deemed only Spearman correlations below the .01 level (2-tailed) to be significant. Differences measured with the Mann-Whitney *U* test were assumed to be significant only below the .017 (Bonferroni correction: .05/3) level.

An a priori sample size calculation was conducted in G*Power (version 3.1.9.2; http://www.gpower.hhu.de/) with a Cohen *d* effect size of 0.40, a significance level of .05, and a desired power of .90 (*F* tests; 1-way analysis of variance). The Cohen *d* effect size was calculated on the basis of PPT data reported in previous studies [34, 67] for patients with CWAD, patients with CINP, and people who were healthy and pain-free. The calculation revealed that a total sample size of at least 84 participants, with 28 participants per group, was required.

Results

Differences between participants with CWAD and CINP and healthy controls

Demographic characteristics and self-reported pain and disability measures

The demographic characteristics and self-reported pain and disability measures are shown in Table 1, and test statistics are shown in appendix A. All study groups were comparable in age, body height, body weight, body mass index, and education level. Furthermore, both groups with chronic neck pain were comparable in neck pain duration, current neck pain intensity, and frequency of neck pain complaints per week.

Disability

The NDI scores were significantly higher in both groups with chronic neck pain compared to healthy controls ($p<.001$). Participants with CWAD reported significantly more pain-related disability compared to participants with CINP ($p<.001$). Furthermore, compared with controls, both groups with chronic neck pain had diminished health-related quality of life (SF-36 total) ($p<.001$). Both physical health-related quality of life and mental health-related quality of life were reduced ($p<.001$). Significantly lower health-related quality of life (lower scores on SF-36 total, mental health, and physical health) was observed in participants with CWAD compared to participants with CINP ($p<.017$).

Cognitive performance

The results for self-reported cognitive deficits (mPDQ total score and subscale scores) and the objective cognitive performance test (TMT parts A and B) are shown in Figure 2, Table 1, and appendix A.

Compared with women who were healthy, participants with CINP or CWAD reported more self-perceived cognitive deficits (mPDQ total score) ($p<.017$ or $p<.001$, respectively). Compared with controls, participants with CWAD reported more cognitive deficits on all mPDQ subscales ($p<.001$), whereas participants with CINP reported more cognitive deficits on only 2 subscales of the mPDQ (attention-concentration and organisation-planning) ($p<.017$). Post hoc paired comparisons indicated significantly more attention-concentration problems ($p<.001$), retrospective memory problems ($p<.001$), and prospective memory problems ($p<.017$) and more severe deficits in total subjective cognitive performance ($p<.001$) in participants with CWAD compared to those with CINP.

The time needed to perform TMT part A and TMT part B was significantly longer in participants with CWAD compared to women who were healthy ($p<.017$). In addition, the time needed to perform TMT part A was significantly longer in participants with CWAD than in those with CINP ($p<.017$).

Table 1 Demographic characteristics, self-reported pain and disability measures, subjective cognitive performance, objective cognitive performance, local and distant hyperalgesia and conditioned pain modulation efficacy.

			Mean	Median	Range (min-max)	IQR	SD	*P-value*	*P-value post-hoc*
Demographic characteristics	**Age (y)**[a]	***CON***	31.96	25.50	19.00 - 62.00	23.00 – 42.00	13.36	0.142	NA
		CINP	35.66	35.00	18.00 – 54.00	27.00 – 45.00	10.80		
		CWAD	36.00	36.50	21.00 – 58.00	25.00 – 44.00	10.79		
	Body height (cm)[b]	***CON***	167	167	155 - 178	163 - 170	6	0.786	NA
		CINP	167	168	157 - 175	163 - 170	5		
		CWAD	168	167	158 – 176	163 – 173	5		
	Body mass (kg)[a]	***CON***	61.24	60.00	51.00 – 81.00	56.25 - 66.00	6.97	0.600	NA
		CINP	63.81	61.50	50.00 – 86.00	56.75 – 70.50	9.22		
		CWAD	62.98	62.50	48.00 – 95.00	53.00 – 69.00	11.71		
	Body mass Index (kg/m²)[a, †]	***CON***	21.87	21.83	18.07 – 26.75	20.62 – 23.33	2.00	0.461	NA
		CINP	22.81	22.74	18.65 – 29.07	20.31 – 24.53	2.76		
		CWAD	22.37	22.31	16.65 – 32.05	19.29 – 23.83	3.80		
			Frequencies						
	Education level (n)[c] No degree; lower secon.; Higher	***CON***	0; 1; 7; 20					1.000	NA
		CINP	0; 2; 8; 23						
		CWAD	1; 2; 7; 22						
Self-reported pain and disability measures	**Neck pain duration (months)**[a]	***CON***	NA	NA	NA	NA	NA	NA	NA
		CINP	84.62	50.00	4.00 – 300.00	24.00 – 120.00	85.35	0.729	
		CWAD	85.17	60.00	3.00 – 444.00	30.50 – 110.00	88.59		
	Days/week neck pain?[a]	***CON***	NA	NA	NA	NA	NA	NA	NA
		CINP	5.26	5.00	3.00 – 7.00	4.00 – 7.00	1.60	0.232	
		CWAD	5.78	7.00	2.00 – 7.00	4.00 – 7.00	1.68		
	Current neck pain intensity (VNRS/10) [a, †]	***CON***	0.00	0.00	0.00 – 0.00	0.00 – 0.00	0.00	**< 0.001**	**< 0.001**[d,e] **0.007**[f]
		CINP	3.88	3.00	1.00 – 8.00	2.00 – 6.00	2.33		
		CWAD	5.66	6.00	1.00 – 10.00	3.25 – 7.00	2.54		
	Neck disability index (/50)[a]	***CON***	2.37	2.00	1.00 – 6.00	1.00 – 4.00	1.50	**< 0.001**	**< 0.001**[d,e,f]
		CINP	16.79	16.50	10.00 – 27.00	12.00 – 21.00	5.04		
		CWAD	22.94	23.00	10.00 – 37.00	18.25 – 27.00	6.58		
	Central sensitization inventory (/100)[a]	***CON***	20.65	21.00	9.00 – 35.00	16.00 – 25.25	6.78	**< 0.001**	**< 0.001**[d,e] **0.004**[f]
		CINP	40.36	39.00	22.00 – 68.00	33.50 – 47.50	10.24		
		CWAD	48.24	49.00	13.00 – 67.00	40.00 – 57.50	12.44		
	SF-36 physical healthy total (/500) [a, †]	***CON***	437.50	440.00	352.50 – 490.00	420.00 – 460.00	33.32	**< 0.001**	**< 0.001**[d, e] **0.003**[f]
		CINP	305.91	330.00	152.50 – 440.00	250.00 – 357.50	74.31		
		CWAD	231.72	211.25	40.00 – 455.00	158.75 – 313.12	104.33		
	SF-36 mental healthy total (/300) [a, †]	***CON***	275.89	284.00	197.33 – 300.00	268.00 – 288.00	22.07	**< 0.001**	**< 0.001**[d, e] **0.003**[f]
		CINP	234.01	247.00	102.50 – 292.00	195.41 – 280.00	55.71		
		CWAD	188.04	213.50	24.00 – 292.00	135.04 – 234.87	70.75		
	SF-36 total (/800)[a, †]	***CON***	713.39	716.00	597.33 – 782.00	695.00 – 745.00	45.14	**< 0.001**	**< 0.001**[d, e] **0.002**[f]
		CINP	539.92	565.66	295.00 – 697.50	470.50 – 629.25	116.51		
		CWAD	419.75	428.58	94.00 – 699.50	302.87 – 537.25	156.50		
Subjective cognitive performance (mPDQ)	**Attention-concentration (/16)**[a, †]	***CON***	3.88	4.00	0.00 – 10.00	2.00 – 5.00	2.14	**< 0.001**	**0.001**[d] **< 0.001**[e, f]
		CINP	6.00	6.00	0.00 – 12.00	4.00 – 7.00	2.69		
		CWAD	9.00	9.00	3.00 – 15.00	7.00 – 11.00	3.14		
	Prospective memory (/20)[a, †]	***CON***	3.04	3.00	0.00 – 7.00	1.00 – 5.25	2.34	**0.001**	0.316[d] **< 0.001**[e] **0.002**[f]
		CINP	4.00	3.00	0.00 – 12.00	2.00 – 5.00	3.04		
		CWAD	6.61	6.00	0.00 – 14.00	4.00 – 9.00	3.79		
	Retrospective memory (/16)[a, †]	***CON***	2.46	2.00	0.00 – 7.00	0.75 – 4.00	2.23	**< 0.001**	0.231[d] **< 0.001**[e, f]
		CINP	3.25	2.00	0.00 – 10.00	1.25 – 5.75	2.63		
		CWAD	6.87	6.00	0.00 – 14.00	4.00 – 10.00	3.90		
	Organization-Planning (/20)[a, †]	***CON***	2.54	2.00	0.00 – 8.00	1.00 – 4.25	2.37	**< 0.001**	**0.004**[d] **< 0.001**[e] 0.017[f]
		CINP	4.84	4.00	0.00 – 12.00	3.00 – 7.75	3.17		
		CWAD	7.48	6.00	1.00 – 17.00	4.00 – 11.00	4.58		
	Total (/72)[a, †]	***CON***	11.92	10.50	1.00 – 27.00	6.00 – 16.25	7.33	**< 0.001**	**0.013**[d] **< 0.001**[e, f]
		CINP	18.09	14.00	5.00 – 44.00	11.25 – 21.75	9.91		
		CWAD	30.10	28.00	6.00 – 57.00	18.00 – 43.00	13.61		

Table 1 Continued.

			Mean	Median	Range (min-max)	IQR	SD	*P-value*	*P-value post-hoc*
Objective cognitive performance (TMT)	**TMT part A (sec)**[a, †]	***CON***	18.30	18.48	10.03 – 29.75	15.64 – 21.26	4.25	**< 0.001**	0.212[d] **< 0.001**[e] **0.001**[f]
		CINP	19.90	19.37	11.56 – 30.13	16.86 – 23.09	4.33		
		CWAD	29.80	27.09	15.06 – 81.00	18.83 – 32.80	14.80		
	TMT part B (sec)[a, †]	***CON***	41.02	34.04	21.44 – 128.00	27.42 – 46.76	22.96	**0.002**	0.095[d] **0.001**[e] 0.017[f]
		CINP	44.88	38.22	26.60 – 148.00	31.45 – 47.74	23.74		
		CWAD	64.13	46.00	25.86 – 251.00	37.02 – 80.00	43.93		
	TMT (B-A)[a]	***CON***	22.72	17.78	2.25 – 98.25	11.68 – 25.30	20.44	0.292	NA
		CINP	24.98	19.00	7.08 – 121.02	14.28 – 24.61	21.68		
		CWAD	34.33	21.00	5.85 – 170.00	13.27 – 39.52	33.98		
	TMT (B/A)[a]	***CON***	2.21	2.05	1.12 – 4.30	1.64 – 2.62	0.80	0.950	NA
		CINP	2.24	1.96	1.33 – 5.49	1.75 – 2.36	0.88		
		CWAD	2.20	1.84	1.19 – 4.89	1.58 – 2.96	0.98		
Local HA	**PPT trapezius (kgf)**[a]	***CON***	4.45	3.85	2.46 – 9.81	3.35 – 5.57	1.74	**< 0.001**	**0.004**[d] **< 0.001**[e] 0.320[f]
		CINP	3.30	3.09	1.18 – 7.43	2.09 – 4.05	1.67		
		CWAD	2.89	2.72	0.13 – 9.30	1.81 – 3.41	1.91		
Distant HA	**PPT hand (kgf)**[a]	***CON***	3.96	3.33	2.30 – 9.80	2.70 – 5.13	1.77	**0.040**	0.268[d] **0.016**[e] 0.095[f]
		CINP	3.48	3.12	1.26 – 8.79	2.36 – 4.42	1.58		
		CWAD	2.72	2.84	0.12 – 5.62	1.67 – 3.65	1.34		
	PPT low back (kgf)[a]	***CON***	5.12	4.26	2.67 – 12.01	3.61 – 6.00	2.32	**0.007**	0.399[d] **0.002**[e] 0.023[f]
		CINP	4.52	4.24	1.65 – 9.75	2.64 – 5.51	2.03		
		CWAD	3.40	3.05	0.50 – 8.06	1.72 – 4.70	1.98		
	PPT quadriceps (kgf)[b]	***CON***	5.04	4.86	2.94 – 8.40	3.84 – 5.87	1.46	**0.006**	0.157[d] **0.005**[e] 0.487[f]
		CINP	4.13	3.79	1.45 – 9.72	2.54 – 5.68	2.02		
		CWAD	3.51	3.52	0.30 – 7.72	2.03 – 4.74	1.86		
Efficacy of CPM	**CPM trapezius** (PPT trapezius during CPT minus PPT trapezius before CPT)[a]	***CON***	1.06	0.94	- 0.18 – 3.45	0.15 – 1.72	0.96	0.086	0.452[d] 0.033[e] 0.125[f]
		CINP	0.91	0.73	- 0.72 – 4.18	0.07 – 1.53	1.09		
		CWAD	0.46	0.32	- 1.26 – 2.69	- 0.05 – 1.02	0.82		
	CPM quadriceps (PPT quadriceps during CPT minus PPT quadriceps before CPT)[b]	***CON***	1.17	1.30	- 0.14 – 3.00	0.68 – 1.49	0.68	**0.003**	1.000[d] **0.004**[e] **0.015**[f]
		CINP	1.05	0.90	- 0.59 – 3.29	0.39 – 1.66	1.01		
		CWAD	0.41	0.30	- 1.05 – 1.87	- 0.10 – 1.03	0.82		

[a]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and the Mann-Whitney U test for post-hoc comparisons. Shapiro-Wilk test $p<0.05$ and visual inspection of the QQ-plot and histogram provided information that the data were not normally distributed. To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.017 level (Bonferonni correction: 0.05/3).
[b]= Data which were assumed to be normally distributed were analyzed with the one-way ANOVA test and post-hoc pairwise comparisons were applied using Bonferroni correction. [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. [†]Variances were not equally distributed across groups, Levene's test p<0.05
[d]= *p*-value for significant differences between CON-CINP, [e]= *p*-value for significant differences between CON-CWAD, [f]= *p*-value for significant differences between CINP-CWAD. Abbreviations: y= years. CON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain. VNRS= verbal numeric rating scale. SF-36= Short Form Health Survey, No degr= no degree, Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, HA= hyperalgesia, CPM= conditioned pain modulation, CPT= cold pressor test, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, kgf= kilogram force, PPT= pressure pain thresholds, VNRS= verbal numeric rating scale, IQR= interquartile range, NA= not applicable

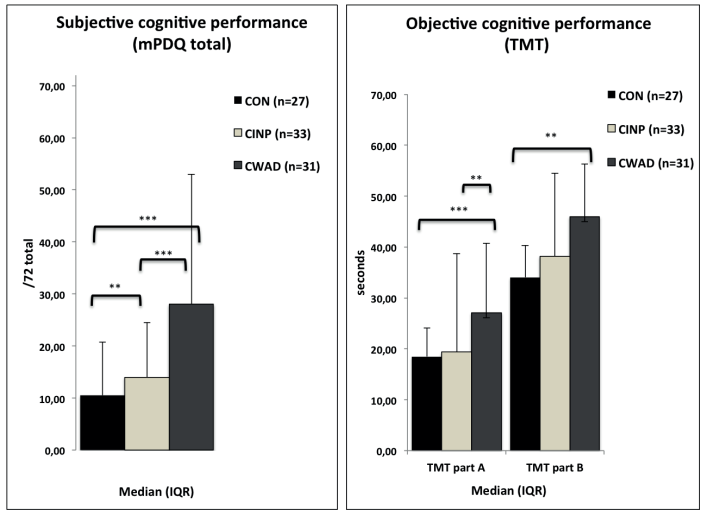


Figure 2 Cognitive performance

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, IQR= interquartile range, mPDQ= modified Perceived Deficits Questionnaire, TMT= Trail Making Test.
Median and IQR are presented when the Kruskal-Wallis Test (post-hoc Mann-Whitney U) was applied for data observed as not normally distributed; **=p<0.017, ***=p<0.001.

Executive function, examined by calculating the difference between scores on TMT B and scores on TMT A, and cognitive flexibility, examined by calculating the ratio of scores on TMT B to scores on TMT A, revealed no significant differences among all study groups (p>.05).

Central sensitization

The outcomes of the measurements of central sensitization are shown in Table 1 and appendix A (self-perceived symptoms) and in Table 1, appendix A, and Figures 3 and 4 (experimental measures).

Self-perceived central sensitization symptoms

Both groups with chronic neck pain reported significantly more self-perceived central sensitization symptoms than women who were healthy and pain-free (p<.001), and participants with CWAD experienced significantly more central sensitization symptoms than participants with CINP (p<.017).

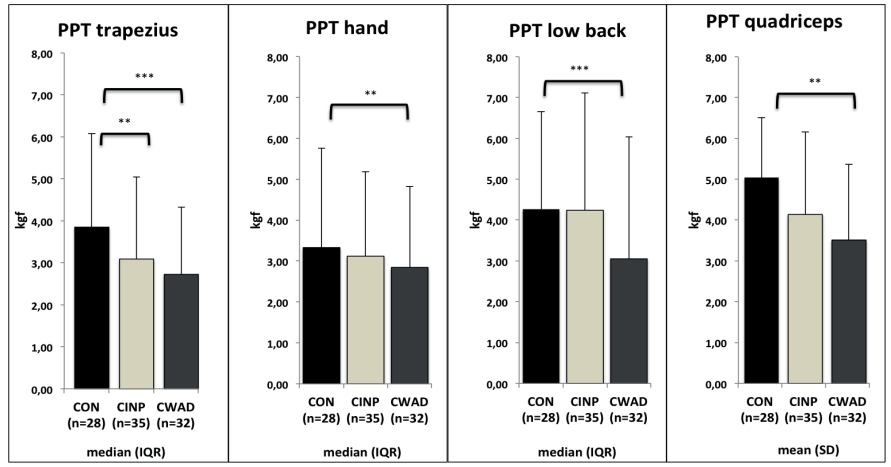


Figure 3 Local and Distant Hyperalgesia

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, PPT= pressure pain thresholds, CPM= conditioned pain modulation, kgf = kilogram force (1 kgf = 9,81 N), IQR=interquartile range, **=p<0.017, ***=p<0.001, Mean and SD are presented when One-way ANOVA (post-hoc bonferroni) was applied for data observed as normally distributed. Median and IQR are presented when Kruskal-Wallis Test (post-hoc Mann-Whitney U test) was applied for data observed as not normally distributed. Results of experimental measures of central sensitization are presented in particular pressure pain threshold measurements to examine local hyperalgesia (PPT trapezius) and distant hyperalgesia (PPT hand, PPT low back, PPT quadriceps).

=<0.017: Kruskal-Wallis/Mann-Withney; *=<0.001: One-way ANOVA (bonferroni);
CON= healthy pain free controls; CINP= chronic idiopathic neck pain;
CWAD= chronic whiplash associated disorders; PPT= pressure pain thresholds

Pressure pain hyperalgesia

Decreased PPTs were demonstrated at the middle trapezius muscle, quadriceps muscle, hand, and lumbar region in participants with CWAD but only at the middle trapezius muscle in participants with CINP, relative to the results for women who were healthy (p<.017). No significant differences between participants with CWAD and participants with CINP were found for PPTs at the 4 locations.

Conditioned Pain Modulation

The CPM values (PPT during cold pressor test minus PPT before cold pressor test) measured at the quadriceps muscle were significantly lower in participants with CWAD than in women who were healthy and participants with CINP (p<.017). The CPM values measured

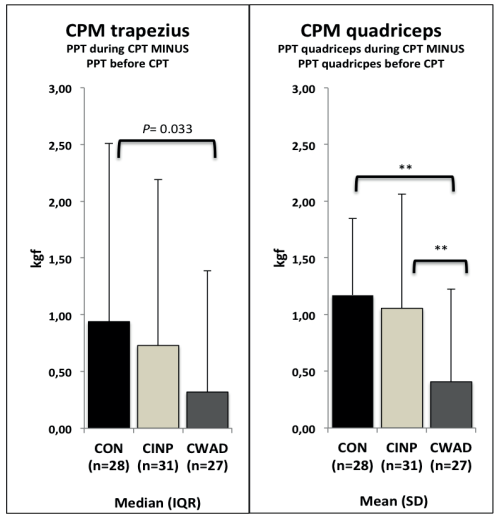


Figure 4 Efficacy of Conditioned Pain Modulation

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, CPM= conditioned pain modulation, kgf = kilogram force (1 kgf = 9,81 N), IQR=interquartile range, CPT= cold pressor test, **=p<0.017, ***=p<0.001, Mean and SD are presented when One-way ANOVA (post-hoc bonferroni) was applied for data observed as normally distributed. Median and IQR are presented when Kruskal-Wallis Test (post-hoc Mann-Whitney U test) was applied for data observed as not normally distributed. Results of experimental measures of central sensitization are presented, in particular measurements of CPM efficacy at the middle trapezius and at the quadriceps muscle.

=<0.017: Kruskal-Wallis/Mann-Withney; *=<0.001: One-way ANOVA (bonferroni);
CON= healthy pain free controls; CINP= chronic idiopathic neck pain;
CWAD= chronic whiplash associated disorders; PPT= pressure pain thresholds

at the middle trapezius muscle were not significantly different among all study groups (p>.017).

Three participants with CINP and 5 participants with CWAD removed their hands from the water before the end of the 2 minutes during the cold pressor test.

Interrelationships among disability, cognitive deficits, and central sensitization

Relationships between subjective or objective cognitive performance and experimental measures of central sensitization

The results of the Spearman correlation (ρ) analyses of subjective or objective cognitive performance and experimental measures of central sensitization are shown in Table 2. These analyses revealed significant moderate negative relationships between PPTs at local symptomatic or distant asymptomatic locations (ρ= -.456 to ρ= -.688) and self-reported cognitive performance in participants with CWAD (p<.001) and between the PPT at the local region (ρ= -.463) and self-reported cognitive performance in participants with CINP (p<.01). Only in participants with CWAD did Spearman correlation analyses demonstrate significant moderate negative relationships (ρ= -.460 to ρ= -.485) between PPTs at local or distant locations and objective cognitive performance, in particular visual attention and visual-motor speed (TMT part A) (p<.01). In contrast, no significant correlations were found between self-perceived or objectively measured cognitive performance and CPM efficacy in both groups of participants with chronic neck pain.

Relationships between disability and experimental measures of central sensitization

The results of the Spearman correlation analyses of self-reported disability characteristics and experimental measures of central sensitization are shown in Table 2. In both groups of participants with chronic neck pain, no significant correlations were found between PPTs or CPM efficacy and neck pain-related disability. Only in participants with CWAD was health-related quality of life found to be significantly moderately correlated (ρ= .459) with the PPT at the middle trapezius muscle (p<.01). The latter correlation was not observed in participants with CINP.

Relationships between disability and subjective or objective cognitive performance

The results of the Spearman correlation analyses of self-reported disability characteristics and cognitive performance are shown in Table 3. Participants with CWAD had significant moderate to strong positive relationships (ρ= .486 to ρ= .637) between neck pain-related disability and self-perceived cognitive deficits or objectively measured cognitive performance. Higher NDI scores correlated with more deficits on the mPDQ (p<.001) and more deficits on the TMT parts A and B (p<.01). Furthermore, in participants with CWAD, significant moderate to strong negative correlations (ρ= -.470 to ρ= -.701) were observed between health-related quality of life and mPDQ total scores (p<.001) or the TMT parts A and B (p<.01).

Table 2 Correlations between **cognitive performance and disability characteristics,** and **experimental measures of central sensitization** in patients with CINP and CWAD.

	PPT trapezius	PPT quadriceps	PPT low back	PPT hand	CPM trapezius	CPM quadriceps
CINP (n=35)						
Self-perceived cognitive performance (mPDQ)						
Total score	**-.463** **.008**	-.238 .189	-.390 .028	-.198 .276	-.033 .867	-.224 .253
Objective cognitive performance (TMT)						
Part A	-.170 .345	-.105 .560	-.126 .486	-.376 .031	-.234 .223	.137 .479
Part B	-.119 .509	.130 .471	-.003 .987	-.033 .854	.046 .811	.029 .881
Disability characteristics						
Pain-related Disability	-.312 .107	-.379 .046	-.256 .189	-.237 .224	.023 .916	-.085 .694
Health-related QoL total	.301 .088	.290 .090	.299 .307	.183 .101	.148 .455	.097 .619
CWAD (n=32)						
Self-perceived cognitive performance (mPDQ)						
Total score	**-.679***** **< .001**	**-.595***** **< .001**	**-.516**** **.003**	**-.688***** **< .001**	.042 .837	-.334 .095
Objective cognitive performance (TMT)						
Part A	**-.485**** **.006**	**-.456**** **.010**	-.369 .041	**-.460**** **.009**	.069 .739	-.335 .094
Part B	-.265 .149	-.259 .159	-.164 .379	-.344 .058	.179 .382	.223 .272
Disability characteristics						
Pain-related Disability	-.337 .060	-.346 .052	-.409 .020	-.366 .039	.126 .530	-.135 .501
Health-related QoL total	**.459**** **.008**	.293 .103	.203 .265	.375 .034	-.126 .532	.089 .657

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons.
** Spearman correlation is significant at the 0.01 level (2-tailed). *** Spearman correlation is significant at the 0.001 level (2-tailed). *P*-values are presented below the correlation coefficient. CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, mPDQ= modified Perceived Deficits Questionnaire, TMT= Trail Making Test, PPT= Pressure Pain Thresholds, CPM= Conditioned Pain Modulation, PPT= Pressure Pain Thresholds, CPM= Conditioned Pain Modulation, QoL= quality of life.

Table 3 Correlations between **disability characteristics** and **cognitive performance** in CINP and CWAD patients.

	mPDQ Total	TMT Part A	TMT Part B
CINP (n=35)			
Disability characteristics			
Pain-related Disability	.279 .159	.001 .997	.197 .315
Health-related QoL Total	-.345 .057	-.076 .680	-.074 .686
CWAD (n=32)			
Disability characteristics			
Pain-related Disability	**.637***** **< .001**	**.556***** **.001**	**.486**** **.006**
Health-related QoL Total	**-.701***** **< .001**	**-.577***** **.001**	**-.470**** **.008**

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons.
** Spearman correlation is significant at or below the 0.01 level (2-tailed). *** Spearman correlation is significant at or below the 0.001 level (2-tailed). *P*-values are presented below the correlation coefficient. CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, mPDQ= modified Perceived Deficits Questionnaire, TMT= Trail Making Test.

Discussion

The first goal of the present case-control study was to examine differences in disability, cognitive deficits, and central sensitization between participants with traumatic chronic neck pain and CINP and women who were healthy and pain-free. The second goal was to investigate relationships among disability, cognitive deficits, and experimental measures of central sensitization in both chronic neck pain conditions.

The results of the present study provided evidence for pain-related disability, reduced physical health- and mental health-related quality of life, and self-perceived cognitive deficits in participants with CWAD and CINP, relative to the results for women who were healthy. Moreover, participants with CWAD reported more deficits in all of these parameters than participants with CINP. Consistent with the findings of the present study, several other studies found cognitive deficits in people with CWAD, relative to the results for people who were healthy [13, 32, 68]. For example, Sullivan et al. observed subjective cognitive deficits in patients with CWAD compared to healthy persons [68]. Furthermore,

we demonstrated decreased objective cognitive performance only in participants with CWAD, relative to the results for women who were healthy. Similarly, 2 recent studies revealed significant objective cognitive deficits in patients with CWAD, compared with the results for volunteers who were healthy [13, 32]. To our knowledge, a comparison of subjective and objective cognitive deficits in people with CINP and people with CWAD has not been reported in the literature. Hence, the present study adds novel and valuable insights into the symptomatology of these conditions.

Additionally, as evidenced by the CSI results, significantly more self-perceived symptoms suggestive of central sensitization were found in both groups of participants with chronic neck pain than in controls. Nevertheless, a CSI score of 40 of 100 best distinguished between people who had a central sensitivity syndrome (CSI scores of ≥ 40/100) and people who did not have a central sensitivity syndrome (sensitivity = 81%, specificity = 75%) [54]. We found a median CSI score of 49 of 100 for participants with CWAD; this score suggested the presence of central sensitization in most participants with CWAD. In contrast, participants with CINP had a median CSI score of 39; this score was below the cutoff and did not imply the presence of central sensitization in most participants with CINP. To the best of our knowledge, the present study is the first to assess these relevant self-perceived central sensitization symptoms in people with CINP or CWAD; therefore, comparisons with other studies are difficult.

Pressure pain thresholds measured at the middle trapezius muscle were not different in the 2 groups of participants with chronic neck pain. However, compared with women who were healthy, participants with CWAD or CINP had increased sensitivity for pressure stimuli at the symptomatic middle trapezius muscle, reflecting local hyperalgesia. These results are in accordance with those of the study of Scott et al [34], who demonstrated local hyperalgesia in both patients with CINP and patients with CWAD, relative to the results for volunteers who were healthy. Comparably, Chien and Sterling [35] showed lower PPTs at the cervical region in both patients with CWAD and patients with CINP than in controls; in addition, they did not observe differences in PPTs at this local region between the 2 groups with chronic neck pain.

In the present study, compared with women who were healthy, participants with CWAD had increased sensitivity for pressure stimuli at distant regions, reflecting distant hyperalgesia and indicating significant signs of central sensitization and, thus, neuroplastic changes in the central nervous system. Likewise, Scott et al [34] reported decreased PPTs over the tibialis anterior muscle and median and radial nerve trunks in patients with CWAD but not in patients with CINP. Additionally, in the present study, decreased CPM efficacy, as assessed with the cold pressor test, was observed only in participants with CWAD; this finding implied disturbed endogenous pain inhibitory mechanisms in these participants and not in participants with CINP. In accordance with these findings, Chua et al [69] could not detect less effective inhibitory modulation in the trigeminal and spinal sensory systems in patients with CINP. Furthermore, the lack of signs of central sensitization in participants with CINP is consistent with the conclusion of a systematic review that central sensitization is not a characteristic feature of CINP [36].

Remarkably, the results of the self-reported CSI were different from the results of the experimental measures of central sensitization because, compared with women who were healthy, both participants with CINP and participants with CWAD reported significantly more central sensitization symptoms, whereas experimentally measured central sensitization was present only in participants with CWAD. The strength and the novelty of the present study compared with previous research are that the presence of central sensitization was examined by combining 3 important central sensitization measures: self-reported central sensitization symptoms (CSI), distant hyperalgesia (PPTs), and efficacy of endogenous pain inhibition (CPM). Our results add important evidence that central sensitization is present, at a group level, in people with CWAD but not in people with CINP.

Regarding the relationships among disability, cognitive performance, and central sensitization, the present study revealed that increased local hyperalgesia and distant hyperalgesia were moderately to strongly correlated with more subjective and objective cognitive deficits in participants with CWAD. On the contrary, CPM efficacy was not correlated with cognitive performance. This finding is in contrast to our hypothesis that the malfunctioning of endogenous pain inhibition and subsequent chronic pain could be associated with decreased cognitive performance. However, another recent study also did not reveal significant correlations between decreased cognitive performance and CPM efficacy in patients with CWAD [13]. Furthermore, consistent with our findings, Meeus et al [32] did not find correlations between CPM efficacy and objective cognitive performance in people with CWAD.

In participants with CWAD, higher pain-related disability and reduced health-related quality of life were moderately to strongly correlated with increased subjective cognitive deficits and decreased performance on the objective cognitive test. However, in participants with CINP, positive moderate correlations were observed only for self-perceived cognitive deficits and local hyperalgesia at the middle trapezius muscle. Furthermore, the results obtained for participants with CWAD were in accordance with a recent study that provides evidence for significant correlations between decreased attention and working memory capacities (computer-based tests), and reduced health-related quality of life in people who were healthy, patients with CWAD, and patients with fibromyalgia [13]. Our results provide additional evidence for the latter relationship

between reduced quality of life and increased objective cognitive deficits in people with CWAD. The strength of the present study was the use of the TMT, which can be quickly and easily administered in clinical practice, as an objective cognitive test.

In summary, significant correlations among disability, cognitive performance, and experimental measures of central sensitization were demonstrated in participants with CWAD. However, in participants with CINP, significant correlations were demonstrated only for subjective cognitive deficits and local hyperalgesia. These observations reflected differences between the groups of participants with chronic neck pain at the level of interrelationships among the assessed variables and thus indicated different underlying mechanisms.

Limitations and Strengths

With regard to interpretation of the results of the present study, the following limitations must be taken into account. First, the cross-sectional nature of the study implied that no conclusions about the causality of the observed correlations can be drawn. Furthermore, the generalizability of the study results may be reduced because only women and only those with CWAD classifiable as WAD II A, B, or C were included. Finally, with regard to interpretation of the cognitive test results, the possible presence of malingering or feigned cognitive deficits in women with CINP and, in particular, women with CWAD must be considered.

However, the present study also had several strengths. First, to our knowledge, this study is the first to address these clinically relevant research questions regarding differences between CWAD and CINP. Second, numerous significant moderate to strong correlations (ρ= .456 to ρ= .70) among disability, cognitive deficits, local hyperalgesia, and distant hyperalgesia were observed in this study.

An important third strength of the present study was the comparability of the groups in age, sex, body mass index, and education level. Neck pain duration and current neck pain intensity were comparable for the groups as well. Another important strength of the present study was that the researchers anticipated sources of bias, such as use of medications, caffeine, alcohol, and nicotine; pregnancy; and performance of heavy physical exertion on the assessment day. A final strength was that the sample sizes for both groups with chronic neck pain and for controls were large compared with those in previous studies in this research domain.

Clinical message and recommendations for further research

The findings of the present study indicate that CWAD and CINP are separate clinical conditions, with similar but also prominently different underlying mechanisms. In particular, pain-related disability, decreased physical health- and mental health-related quality of life, and self-reported cognitive deficits were demonstrated in participants with CWAD and, to a significantly lesser extent, in participants with CINP. However, decreased objective cognitive performance was present only in participants with CWAD. Local pressure hyperalgesia at the middle trapezius muscle was present in both groups of participants with chronic neck pain. In contrast, distant hyperalgesia and decreased CPM efficacy were present in participants with CWAD but not in those with CINP, indicating the presence of central sensitization only in participants with CWAD. Therefore, the results provide preliminary evidence for the clinical importance of distinguishing assessment and rehabilitation approaches for patients with traumatic chronic neck pain and those with CINP.

On the basis of the results of the present study, we recommend that disability, self-perceived and objective cognitive deficits, signs of central sensitization, and their possible interrelationships should be evaluated with the aim of providing more effective and individually tailored therapy. In view of the observed correlations with local hyperalgesia and distant hyperalgesia in participants with CWAD, targeting the modification of disability and cognitive deficits should be an integral part of therapy for patients with CWAD. Questionnaires such as the NDI, the SF-36, and the mPDQ could be used to evaluate and re-evaluate respectively disability, health-related quality of life, and cognitive deficits in both patients with CINP and patients with CWAD. Furthermore, the TMT could be used as an objective test to examine cognitive performance in patients with CWAD.

Additionally, therapists should be aware of the possibility of central sensitization in patients with CWAD. Therefore, therapists should be able to recognize central sensitization. Clinical guidelines for recognizing and treating central sensitization are available in current literature [70, 71]. The evaluation of self-reported central sensitization symptoms, distant hyperalgesia, and endogenous pain inhibition can contribute to the recognition of central sensitization. When the clinical picture is characterized and dominated by central sensitization or when maladaptive illness perceptions are present [72], neurophysiological pain education should be applied. The goals of such education are to change inappropriate pain beliefs, reduce maladaptive attitudes, cognitions, and behavior in relation to pain; and subsequent increase participation in active treatment [73, 74]. This type of pain education will generally be more applicable for patients with CWAD, but it may be relevant for patients with CINP because, at an individual patient level, it is still possible that central sensitization is present in patients with CINP. Previous research in patients with CWAD demonstrated significant decreases in pain-related disability and increases in PPTs after pain neurophysiology education [74].

On the basis of the results of the present study, cognitive deficits, such as attention, concentration, and memory problems, should be taken into account in the application of pain education. In patients with acute and subacute whiplash injuries, early pain education

and cognitive behavioural therapy are important for preventing the transition to chronicity [75]. Additionally, cognitive behavioural therapy for patients with CWAD can decrease pain-related disability and post-traumatic stress and increase health-related quality of life [21, 76]. Furthermore, when treating patients with CINP and, in particular, patients with CWAD, physical therapists could apply - as part of their therapy - pain education followed by cognition-targeted motor control training [73].

The results of the present study suggest that the traumatic origin of chronic neck pain in patients with CWAD plays a significant negative role in the clinical symptoms of these patients, relative to the situatin for patients with CINP. The treatment of cervical dysfunctions such as decreased movement control in patients with CWAD or CINP seems plausible, but caution is required not to induce or aggravate pain and other symptoms during the treatment of patients with CWAD [21]. When applying hands-on therapies in patients with dominant central sensitization pain, physical therapists should remember that therapeutic interventions triggering more pain will serve as a new peripheral source of nociceptive input and thus will sustain the central sensitization process [77].

The socioeconomic and psychosocial burdens related to chronic neck pain, the high rate of transition to chronicity, and the limited effects of conservative therapy on pain and disability make current and future research highly valuable. The results of the present study can have implications for health policymakers' decision-making regarding funding and treatment options. Because the present study revealed important correlations, longitudinal studies are recommended to unravel the cause-effect relationships among disability characteristics, subjective or objective cognitive performance, and (central) pain measures in patients with traumatic chronic neck pain and CINP. Additionally, magnetic resonance imaging studies investigating the roles of possible structural and functional brain alterations in the observed dysfunctions could be highly valuable to increase insight into these pain conditions, as it is known that there is an overlap of brain regions involved in the processing of cognitive, affective, and nociceptive information [78, 79]. It would be interesting to explore whether brain alterations are present in these regions in patients with CINP or CWAD and whether such alterations are related to disability, cognition, and clinical or experimental correlates of pain.

Conclusion

In conclusion, this research revealed important differences between women with traumatic chronic neck pain and women with CINP and thus provided evidence of the clinical importance of distinguishing assessment and rehabilitation approaches for these chronic neck pain conditions. Local hyperalgesia was demonstrated in participants with traumatic chronic neck pain and participants with CINP. Distant hyperalgesia and decreased CPM efficacy were shown in participants with CWAD but not in those with CINP; this finding was indicative of the presence of central sensitization in participants with CWAD. Additionally, this research demonstrated disability and cognitive deficits in participants with CWAD and, to a significantly lesser extent, in participants with CINP. Furthermore, moderate to strong positive correlations were observed among disability, cognitive deficits, and local hyperalgesia and distant hyperalgesia in participants with CWAD. However, moderate positive correlations were observed only between self-perceived cognitive deficits and local hyperalgesia in participants with CINP.

We recommend that disability, cognitive deficits, central sensitization, and their inter-relationships should be evaluated with the goal of providing individually tailored therapy targeting the observed deficits.

Acknowledgements

The authors gratefully acknowledge the participants of this study, without whom this study could never have been completed successfully. For its financial support, the authors' gratitude goes out to the Special Research Fund of Ghent University (BOF-Ghent: BOF13/DOC/276) and the Agency for Innovation by Science and Technology (IWT), who made this experimental research possible.

Appendix A Overview of test statistics.

	3 GROUPS		CON – CINP		CON – CWAD		CINP – CWAD	
Demographic characteristics								
Test statistics	***X²***	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***
Age[a]	3.905	0.142	369.500	0.095	327.00	0.073	553.00	0.930
Body mass[a]	1.022	0.600	NA	NA	NA	NA	NA	NA
Body mass index[a]	1.548	0.461	NA	NA	NA	NA	NA	NA
Test statistics	***F***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***
Body height[b]	0.242	0.786	0.53 ± 1.34	1.000	- 0.37 ± 1.35	1.000	- 0.90 ± 1.30	1.000
	Fisher's exact test (2-sided)							
Education level (n) No degree; Lower second.; Higher second.; Higher edu.[a]	2.337	1.000	NA	NA	NA	NA	NA	NA
Self-reported pain and disability measures								
Test statistics	***X²***	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***
Neck pain duration[a]	NA	NA	NA	NA	NA	NA	440.00	0.729
Days/week neck pain?[a]	NA	NA	NA	NA	NA	NA	213.50	0.232
Current neck pain intensity[a]	64.177	**< 0.001**	0.000	**< 0.001**	0.000	**< 0.001**	346.000	**0.007**
Neck disability index[a]	61.282	**< 0.001**	0.000	**< 0.001**	0.000	**< 0.001**	212.50	**< 0.001**
Central sensitization Inventory[a]	50.473	**< 0.001**	34.500	**< 0.001**	28.50	**< 0.001**	273.00	**0.004**
SF-36[a]								
- Physical health total	53.612	**< 0.001**	36.50	**< 0.001**	26.00	**< 0.001**	298.00	**0.003**
- Mental health total	32.419	**< 0.001**	201.00	**< 0.001**	86.50	**< 0.001**	303.00	**0.003**
- SF-36 total	54.960	**< 0.001**	38.50	**< 0.001**	17.00	**< 0.001**	291.00	**0.002**
Subjective cognitive performance (mPDQ)[a]								
Attention-concentration	34.10	**< 0.001**	212.50	**0.001**	69.00	**< 0.001**	237.50	**< 0.001**
Prospective memory	15.15	**0.001**	352.50	0.316	185.50	**< 0.001**	275.50	**0.002**
Retrospective memory	22.54	**< 0.001**	340.50	0.231	139.50	**< 0.001**	224.50	**< 0.001**
Organization-planning	20.957	**< 0.001**	233.00	**0.004**	138.50	**< 0.001**	323.50	0.017
mPDQ total	29.678	**< 0.001**	257.500	**0.013**	92.00	**< 0.001**	255.50	**< 0.001**
Objective cognitive performance (TMT)[a]								
TMT part A	18.16	**< 0.001**	361.50	0.212	173.00	**< 0.001**	264.00	**0.001**
TMT part B	12.44	**0.002**	333.00	0.095	211.50	**0.001**	334.00	0.017
TMT (B-A)	2.46	0.292	NA	NA	NA	NA	NA	NA
TMT (B/A)	0.95	0.621	NA	NA	NA	NA	NA	NA
Local HA								
PPT middle trapezius[a]	15.38	0.000	281.00	0.004	192.50	0.000	481.00	0.320
Distant HA								
PPT hand[a]	6.44	**0.040**	410.00	0.268	286.00	**0.016**	427.00	0.095
PPT low back[a]	10.02	**0.007**	429.00	0.399	242.50	**0.002**	379.00	0.023
Test statistics	***F***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***
PPT quadriceps[b]	5.33	**0.006**	0.90 ± 0.46	0.157	1.53 ± 0.47	**0.005**	0.62 ± 0.44	0.487
Efficacy of CPM								
Test statistics	***X²***	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***	**Mann-Whitney U**	***P-value***
CPM trapezius (PPT during CPT minus PPT before CPT)[a]	4.91	0.086	384.50	0.452	251.50	0.033	320.00	0.125
Test statistics	***F***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***	***Post hoc Bonferroni Estimate ± SE***	***P-value***
CPM quadriceps (PPT quadriceps during CPT minus PPT quadriceps before CPT)[b]	6.441	**0.003**	0.11 ± 0.22	1.000	0.76 ± 0.23	**0.004**	0.65 ± 0.22	**0.015**

[a]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and the Mann-Whitney U test for post-hoc comparisons. To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.017 (Bonferroni correction: 0.05/3). [b]= Data which were assumed to be normally distributed were analyzed with the one-way ANOVA test and post-hoc pairwise comparisons were applied using Bonferroni correction. [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. Test-statistics represent the F-statistic for parametric continuous data, the Mann-Whitney U-statistic for continuous non-parametric data and the χ^2-statistic for categorical data. Abbreviations: CON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain. VNRS= verbal numeric rating scale. SF-36= Short Form Health Survey, No degr= no degree, Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, HA= hyperalgesia, CPM= conditioned pain modulation, CPT= cold pressor test, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, kgf= kilogram force, PPT= pressure pain thresholds, VNRS= verbal numeric rating scale.

References

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J* 2006; 15:834-848.
2. Manchikanti L, Singh V, Datta S, Cohen SP, Hirsch JA. Comprehensive review of epidemiology, scope, and impact of spinal pain. *Pain Physician* 2009; 12:E35-70.
3. Borghouts JA, Koes BW, Bouter LM. The clinical course and prognostic factors of non-specific neck pain: a systematic review. *Pain* 1998; 77:1-13.
4. Barnsley L, Lord S, Bogduk N. Whiplash injury. *Pain* 1994; 58:283-307.
5. Hoy DG, Protani M, De R, Buchbinder R. The epidemiology of neck pain. *Best Pract Res Clin Rheumatol* 2010; 24:783-792.
6. Sheather-Reid RB, Cohen ML. Psychophysical evidence for a neuropathic component of chronic neck pain. *Pain* 1998; 75:341-347.
7. Radanov BP, di Stefano G, Schnidrig A, Ballinari P. Role of psychosocial stress in recovery from common whiplash [see comment]. *Lancet* 1991; 338:712-715.
8. Gore DR, Sepic SB, Gardner GM, Murray MP. Neck pain: a long-term follow-up of 205 patients. *Spine (Phila Pa 1976)* 1987; 12:1-5.
9. Rodriquez AA, Barr KP, Burns SP. Whiplash: pathophysiology, diagnosis, treatment, and prognosis. *Muscle Nerve* 2004; 29:768-781.
10. Davis CG. Mechanisms of chronic pain from whiplash injury. *J Forensic Leg Med* 2013; 20:74-85.
11. Seroussi R, Singh V, Fry A. Chronic whiplash pain. *Phys Med Rehabil Clin N Am* 2015; 26:359-373.
12. Sterling M. A proposed new classification system for whiplash associated disorders--implications for assessment and management. *Man Ther* 2004; 9:60-70.
13. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician* 2015; 18:E389-401.
14. Sterner Y, Gerdle B. Acute and chronic whiplash disorders--a review. *J Rehabil Med* 2004; 36:193-209; quiz 210.
15. Wenzel HG, Haug TT, Mykletun A, Dahl AA. A population study of anxiety and depression among persons who report whiplash traumas. *J Psychosom Res* 2002; 53:831-835.
16. Jull G, Kristjansson E, Dall'Alba P. Impairment in the cervical flexors: a comparison of whiplash and insidious onset neck pain patients. *Man Ther* 2004; 9:89-94.
17. Woodhouse A, Vasseljen O. Altered motor control patterns in whiplash and chronic neck pain. *BMC Musculoskelet Disord* 2008; 9:90.
18. Sterling M, Jull G, Kenardy J. Physical and psychological factors maintain long-term predictive capacity post-whiplash injury. *Pain* 2006; 122:102-108.
19. Ettlin T, Schuster C, Stoffel R, Bruderlin A, Kischka U. A distinct pattern of myofascial findings in patients after whiplash injury. *Arch Phys Med Rehabil* 2008; 89:1290-1293.
20. De Pauw R, Coppieters I, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? - A systematic review. *Man Ther* 2015.
21. Nijs J, Van Oosterwijck J, De Hertogh W. Rehabilitation of chronic whiplash: treatment of cervical dysfunctions or chronic pain syndrome? *Clin Rheumatol* 2009; 28:243-251.
22. Sterling M, Jull G, Vicenzino B, Kenardy J. Sensory hypersensitivity occurs soon after whiplash injury and is associated with poor recovery. *Pain* 2003; 104:509-517.
23. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013; 17:299-312.
24. Stone AM, Vicenzino B, Lim EC, Sterling M. Measures of central hyperexcitability in chronic whiplash associated disorder--a systematic review and meta-analysis. *Man Ther* 2013; 18:111-117.
25. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain* 2009; 10:895-926.
26. Daenen L, Nijs J, Roussel N, Wouters K, Van Loo M, Cras P. Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clin Rheumatol* 2013; 32:23-31.
27. Curatolo M, Arendt-Nielsen L, Petersen-Felix S. Evidence, mechanisms, and clinical implications of central hypersensitivity in chronic pain after whiplash injury. *Clin J Pain* 2004; 20:469-476.
28. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP. Central hypersensitivity in chronic pain after whiplash injury. *Clin J Pain* 2001; 17:306-315.
29. Sterling M, McLean SA, Sullivan MJ, Elliott JM, Buitenhuis J, Kamper SJ. Potential processes involved in the initiation and maintenance of whiplash-associated disorders: discussion paper 3. *Spine (Phila Pa 1976)* 2011; 36:S322-329.
30. Vangronsveld K, Peters M, Goossens M, Linton S, Vlaeyen J. Applying the fear-avoidance model to the chronic whiplash syndrome. *Pain* 2007; 131:258-261.
31. Treleaven J, Jull G, LowChoy N. Smooth pursuit neck torsion test in whiplash-associated disorders: relationship to self-reports of neck pain and disability, dizziness and anxiety. *J Rehabil Med* 2005; 37:219-223.
32. Meeus M, Van Oosterwijck J, Ickmans K, Baert I, Coppieters I, Roussel N, Struyf F, Pattyn N, Nijs J. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clin Rheumatol* 2015; 34:545-553.
33. Kessels RP, Aleman A, Verhagen WI, van Luijtelaar EL. Cognitive functioning after whiplash injury: a meta-analysis. *J Int Neuropsychol Soc* 2000; 6:271-278.
34. Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. *Clin J Pain* 2005; 21:175-181.
35. Chien A, Sterling M. Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. *Man Ther* 2010; 15:48-53.
36. Malfliet A, Kregel J, Cagnie B, Kuipers M, Dolphens M, Roussel N, Meeus M, Danneels L, Bramer WM, Nijs J. Lack of evidence for central sensitization in idiopathic, non-traumatic neck pain: a systematic review. *Pain Physician* 2015; 18:223-236.
37. Elliott JM, Pedler AR, Jull GA, Van Wyk L, Galloway GG, O'Leary SP. Differential changes in muscle composition exist in traumatic and nontraumatic neck pain. *Spine (Phila Pa 1976)* 2014; 39:39-47.
38. Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. *J Pain Res* 2015; 8:311-320.
39. Correa JB, Costa LO, de Oliveira NT, Sluka KA, Liebano RE. Central sensitization and changes in conditioned pain modulation in people with chronic nonspecific low back pain: a case-control study. *Exp Brain Res* 2015; 233:2391-2399.
40. Popescu A, LeResche L, Truelove EL, Drangsholt MT. Gender differences in pain modulation by diffuse noxious inhibitory controls: a systematic review. *Pain* 2010; 150:309-318.
41. Woo A, Lechner B, Fu T, Wong CS, Chiu N, Lam H, Pulenzas N, Soliman H, DeAngelis C, Chow EA. Cut points for mild, moderate, and severe pain among cancer and non-cancer. - *Ann Palliat Med 2015 Oct;4(4):176-83 doi: 103978/jissn2224-582020150904:-* 176-183.
42. Vernon H. The Neck Disability Index: state-of-the-art, 1991-2008. *J Manipulative Physiol Ther* 2008; 31:491-502.
43. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine (Phila Pa 1976)* 1995; 20:1s-73s.
44. Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. *J Manipulative Physiol Ther* 1991; 14:409-415.
45. Jorritsma W, de Vries GE, Geertzen JH, Dijkstra PU, Reneman MF. Neck Pain and Disability Scale and the Neck Disability Index: reproducibility of the Dutch Language Versions. *Eur Spine J* 2010; 19:1695-1701.
46. Jorritsma W, de Vries GE, Dijkstra PU, Geertzen JH, Reneman MF. Neck Pain and Disability Scale and Neck Disability Index: validity of Dutch language versions. *Eur Spine J* 2012; 21:93-100.
47. Ware JE SK KM, Gandex B. SF-36 Health Survey Manual and Interpretation Guide. . MA: New England Medical Center, the Health Institute, Boston, 1993.
48. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, Sprangers MA, te Velde A, Verrips E. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. *J Clin Epidemiol* 1998; 51:1055-1068.
49. Takasaki H, Chien CW, Johnston V, Treleaven J, Jull G. Validity and reliability of the perceived deficit questionnaire to assess cognitive symptoms in people with chronic whiplash-associated disorders. *Arch Phys Med Rehabil* 2012; 93:1774-1781.

50. Sanchez-Cubillo I, Perianez JA, Adrover-Roig D, Rodriguez-Sanchez JM, Rios-Lago M, Tirapu J, Barcelo F. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *J Int Neuropsychol Soc* 2009; 15:438-450.
51. Christidi F, Kararizou E, Triantafyllou N, Anagnostouli M, Zalonis I. Derived Trail Making Test indices: demographics and cognitive background variables across the adult life span. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2015; 22:667-678.
52. Kregel J, Vuijk PJ, Descheemaeker F, Keizer D, van der Noord R, Nijs J, Cagnie B, Meeus M, van Wilgen P. The Dutch Central Sensitization Inventory (CSI): Factor Analysis, Discriminative Power and Test-Retest Reliability. *Clin J Pain* 2015.
53. Mayer TG, Neblett R, Cohen H, Howard KJ, Choi YH, Williams MJ, Perez Y, Gatchel RJ. The development and psychometric validation of the central sensitization inventory. *Pain Pract* 2012; 12:276-285.
54. Neblett R, Cohen H, Choi Y, Hartzell MM, Williams M, Mayer TG, Gatchel RJ. The Central Sensitization Inventory (CSI): establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. *J Pain* 2013; 14:438-445.
55. Neblett R, Hartzell MM, Cohen H, Mayer TG, Williams M, Choi Y, Gatchel RJ. Ability of the central sensitization inventory to identify central sensitivity syndromes in an outpatient chronic pain sample. *Clin J Pain* 2015; 31:323-332.
56. Whiteside A, Hansen S, Chaudhuri A. Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 2004; 109:497-499.
57. Meeus M, Roussel NA, Truijen S, Nijs J. Reduced pressure pain thresholds in response to exercise in chronic fatigue syndrome but not in chronic low back pain: an experimental study. *J Rehabil Med* 2010; 42:884-890.
58. Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. *Scand J Rehabil Med* 1999; 31:89-93.
59. Cathcart S, Pritchard D. Reliability of pain threshold measurement in young adults. *J Headache Pain* 2006; 7:21-26.
60. Ylinen J, Nykanen M, Kautiainen H, Hakkinen A. Evaluation of repeatability of pressure algometry on the neck muscles for clinical use. *Man Ther* 2007; 12:192-197.
61. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, Hansson P, Lautenbacher S, Marchand S, Wilder-Smith. Recommendations on terminology and practice of psychophysical DNIC testing. *- Eur J Pain 2010 Apr;14(4):339 doi: 101016/jejpain201002004 Epub 2010 Mar:-* 339.
62. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol* 2010; 23:611-615.
63. Ng TS, Pedler A, Vicenzino B, Sterling M. Less efficacious conditioned pain modulation and sensory hypersensitivity in chronic whiplash-associated disorders in Singapore. *Clin J Pain* 2014; 30:436-442.
64. Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. *Brain Res* 2008; 1230:73-79.
65. Rehberg B, Baars JH, Kotsch J, Koppe P, von Dincklage F. Comparison of trigeminal and spinal modulation of pain and nociception. *Int J Neurosci* 2012; 122:298-304.
66. Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. *Pain Res Manag* 2012; 17:98-102.
67. De Kooning M, Daenen L, Cras P, Gidron Y, Roussel N, Nijs J. Autonomic response to pain in patients with chronic whiplash associated disorders. *Pain Physician* 2013; 16:E277-285.
68. Sullivan MJ, Hall E, Bartolacci R, Sullivan ME, Adams H. Perceived cognitive deficits, emotional distress and disability following whiplash injury. *Pain Res Manag* 2002; 7:120-126.
69. Chua NHL, Timmerman H, Vissers KC, Wilder-Smith OH. Multi-modal Quantitative Sensory Testing in Patients with Unilateral Chronic Neck Pain: An Exploratory Study. *Journal of Musculoskeletal Pain* 2012; 20:292-299.
70. Nijs J, Torres-Cueco R, van Wilgen CP, Girbes EL, Struyf F, Roussel N, van Oosterwijck J, Daenen L, Kuppens K, Vanwerweeen L, Hermans L, Beckwee D, Voogt L, Clark J, Moloney N, Meeus M. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. *Pain Physician* 2014; 17:447-457.
71. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Man Ther* 2010; 15:135-141.

72. Nijs J, Paul van Wilgen C, Van Oosterwijck J, van Ittersum M, Meeus M. How to explain central sensitization to patients with 'unexplained' chronic musculoskeletal pain: practice guidelines. *Man Ther* 2011; 16:413-418.
73. Nijs J, Meeus M, Cagnie B, Roussel NA, Dolphens M, Van Oosterwijck J, Danneels L. A modern neuroscience approach to chronic spinal pain: combining pain neuroscience education with cognition-targeted motor control training. *Phys Ther* 2014; 94:730-738.
74. Van Oosterwijck J, Nijs J, Meeus M, Truijen S, Craps J, Van den Keybus N, Paul L. Pain neurophysiology education improves cognitions, pain thresholds, and movement performance in people with chronic whiplash: a pilot study. *J Rehabil Res Dev* 2011; 48:43-58.
75. Andersen TE, Ravn SL, Roessler KK. Value-based cognitive-behavioural therapy for the prevention of chronic whiplash associated disorders: protocol of a randomized controlled trial. *BMC Musculoskelet Disord* 2015; 16:232.
76. Dunne RL, Kenardy J, Sterling M. A randomized controlled trial of cognitive-behavioral therapy for the treatment of PTSD in the context of chronic whiplash. *Clin J Pain* 2012; 28:755-765.
77. Nijs J, Van Houdenhove B. From acute musculoskeletal pain to chronic widespread pain and fibromyalgia: application of pain neurophysiology in manual therapy practice. *Man Ther* 2009; 14:3-12.
78. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. *Prog Neurobiol* 2011; 93:385-404.
79. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 2015; 87:474-491.

Chapter 4

Decreased regional grey matter volume in women with chronic whiplash associated disorders: relationships with cognitive deficits and disturbed pain processing

Coppieters I. MSc[1, 2], De Pauw R. MSc[1], Caeyenberghs K. PhD[3], Danneels L. PhD[1], Kregel J. MSc[1, 2, 4], Pattyn A. MSc[1], Meeus M. PhD[1, 2, 5, *], Cagnie B. PhD[1, *]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium
[2] Pain in Motion international research group, www.paininmotion.be
[3] School of Psychology, Faculty of Health Sciences, Australian Catholic University, Australia
[4] Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Faculty of Physical Education & Physiotherapy, Free University Brussels, Belgium
[5] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium

*Equally contributed last authors

Pain Physician: accepted for publication 28 April 2017.
SCI_{2015}= 3.40, 5-year impact factor= 3.694, Q2 in anesthesiology (8/31), Q2 in clinical neurology (53/193).

Abstract

Background: Patients with chronic whiplash associated disorders (CWAD) are characterized by pain of traumatic origin, cognitive deficits, and central sensitization (CS). Previous neuroimaging studies revealed altered grey matter volume (GMV) in mild traumatic brain injury patients and chronic pain conditions also characterized by CS. It can therefore be hypothesized that GMV alterations also play a role in the persistent complaints of CWAD. However, brain alterations remain poorly investigated in these patients.

Objectives: This study examined regional GMV alterations in patients with CWAD compared to patients with non-traumatic chronic idiopathic neck pain (CINP), who normally do not show CS at a group level, and healthy controls. Additionally, in both patient groups relationships between regional GMV, and measures of cognition as well as pain processing were assessed.

Study design: This was a cross-sectional case-control study.

Setting: This study was performed at the Department of Rehabilitation Sciences and Physiotherapy of Ghent University in cooperation with the Ghent Institute for Functional and Metabolic Imaging.

Methods: Ninety-three women (28 controls, 34 CINP, 31 CWAD) were enrolled. First, T1-weighted Magnetic Resonance Images (MRI) were acquired to examine GMV alterations in brain regions involved in processing cognition and pain. Next, cognitive performance, pain cognitions, and central sensitization (CS) symptoms were assessed. Finally, hyperalgesia and conditioned pain modulation efficacy were examined.

Results: Regional GMV of the right lateral orbitofrontal cortex, left supramarginal cortex, and left posterior cingulate cortex was decreased in CWAD compared to healthy controls (p=0.023; p=0.012; p=0.047**,** respectively). Additionally, GMV of the right superior parietal cortex and left posterior cingulate cortex was decreased in CWAD compared to CINP patients (p=0.008; p=0.035, respectively). Decreased regional GMV correlated with worse cognitive performance, higher maladapted pain cognitions, CS symptoms, and hyperalgesia in CWAD (r_s= -0.515 to -0.657; p<0.01). In CINP, decreased regional GMV correlated only with worse cognitive performance (r_s= -0.499 to -0.619; p<0.01), and no GMV differences compared with controls could be revealed.

Limitations: No conclusions about the causality of the observed relationships can be drawn.

Conclusions: These results provide the first evidence for reduced GMV in cortical regions involved in processing cognition and pain in patients with CWAD. Accordingly, it is recommended that therapy approaches for CWAD should address the brain and take into account neuroplasticity of the central nervous system.

Introduction

Chronic neck pain is an enormous healthcare problem and one of the most prevalent musculoskeletal pain conditions worldwide [1, 2]. Furthermore, this pain condition is associated with unexplained symptoms, reduced quality of life, and poor therapy outcomes, thus representing an important source of disability [3-6]. Chronic neck pain can be subdivided, on the basis of its etiology, into three categories: specific neck pain, trauma-induced neck pain, and idiopathic (non-traumatic) neck pain. This article focuses on chronic neck pain of a traumatic and an idiopathic non-traumatic nature.

Chronic whiplash associated disorders (CWAD) are characterized by trauma-induced neck pain lasting more than 3 months resulting from a whiplash injury usually originating from a rear-end motor vehicle crash and caused by acceleration-deceleration forces acting on the neck, head, and torso [7, 8]. *Chronic idiopathic non-traumatic neck pain (CINP)* is characterized by neck pain lasting more than 3 months, without the presence of specific pathoanatomical causes.

Based on a paucity of studies comparing patients with CINP and CWAD, indications for different underlying mechanisms can be found [6, 9]. Cognitive deficits [10], maladapted pain cognitions [11], and central sensitization (CS) [12] have been demonstrated in patients with CWAD. While CS is rare in patients with CINP [13], cognitive deficits, and maladapted pain cognitions are present [6, 14], however to a significantly lesser extent compared to patients with CWAD [6, 13].

Remarkably, although it can be hypothesized that structural brain alterations including grey matter volume (GMV) alterations play a role in the persistent and complex complaints of patients with CWAD, studies examining the presence of GM morphological alterations in patients with CWAD compared to patients with CINP are lacking.

Examining the influence of the traumatic acceleration-deceleration injury, the presence of GMV alterations, and exploring relationships between regional GMV and measures of cognition, pain, and CS is important and could increase our insight into the underlying mechanisms of CINP and CWAD, and their possible differences.

During the past decades, a wide range of Magnetic Resonance Imaging (MRI) techniques explored structural brain alterations *in-vivo* in patients with chronic pain [15-17]. This neuroimaging research has shown structural neuroplasticity, which refers to the ability of the brain to reorganize itself and thereby adapt or maladapt its morphology [18]. Subsequently, the role of maladapted brain alterations, including GMV alterations [16-18], has been gradually elucidated in the persistent pain and associated complaints of various chronic pain conditions (e.g. fibromyalgia [19], chronic low back pain [20], temporomandibular disorders [21], chronic pelvic pain syndrome [22]). Especially, GMV alterations in regions involved in cognitive processing and sensory-discriminative, affective and cognitive pain processing have been shown in various chronic pain syndromes, such as fibromyalgia and

chronic low back pain sharing the common pathophysiology of CS [19, 20]. For example, altered GM morphology in the cingulate, insula, and orbitofrontal cortex, precuneus, amygdala, and thalamus, has been found in these patients. Furthermore, alterations in GM morphology are denoted to be related with persistent pain and cognitive symptoms [19-24], which are commonly reported complaints in these chronic pain conditions [10, 25-27]. Besides, these chronic pain patients often show maladapted pain cognitions including pain catastrophizing and hypervigilance [28], which seem to be associated with GM morphology [29]. Research has furthermore demonstrated changes in GMV in patients with mild traumatic brain injury (TBI) [30], where chronic pain is also a common sequel [31, 32]. In addition, similar to patients with chronic pain, mild TBI patients frequently report persistent cognitive complaints [33] accompanied with reduced cognitive performance [34-36].

Based on the outlined evidence, due to the trauma, and because of cognitive deficits [10], maladapted pain cognitions [11], and CS [12] in CWAD patients, it could be hypothesized that alterations in regional GMV are present in patients with CWAD, but not or to a lesser degree in patients with CINP.

To address the current research gap, the first aim was to examine GMV alterations in brain regions involved in cognitive processing, and regions implicated in sensory-discriminative, affective and cognitive pain processing in patients with CINP and CWAD compared to healthy persons. The second aim was to investigate relationships between regional GMV, and cognitive deficits, pain intensity, pain cognitions, local hyperalgesia, and measures of CS in both chronic neck pain conditions.

Distinct regional GMV alterations and significant relationships with measures of cognition, pain, and CS were mainly hypothesized in patients with CWAD compared to CINP and healthy persons. Accordingly, important differences between patients with CINP and CWAD were hypothesized with a negative mediating role of the trauma in CWAD.

Methods

Study design and procedure

This cross-sectional case-control study took place at the Department of Rehabilitation Sciences and Physiotherapy of Ghent University in cooperation with the Ghent Institute for Functional and Metabolic Imaging. The study was performed from February 2014 to September 2015 and was carried out in accordance with the principles of the Declaration of Helsinki. The local Ethics Committee of the Ghent University Hospital (EC/2013/1053) approved the research protocol. All participants were thoroughly informed about the study procedures and signed an informed consent statement prior to study enrolment.
First, all participants completed a survey to acquire information on demographics, and completed a series of questionnaires to obtain information on disability, pain intensity, pain cognitions, and CS symptoms (as described below). Subsequently, assessments to investigate cognitive deficits and pain processing were performed. On a separate test day (10 +/- 7 days apart), high-resolution T1-weighted MR images and T2*-weighted images of the brain were acquired.

Participants

Ninety-three female participants - 34 patients with CINP, 31 patients with CWAD and 28 healthy pain-free controls - were enrolled in the present study. In order to exclude the confounding factor of sex, we included only women, as research has demonstrated significant differences between men and women regarding GMV, pain sensitivity and pain processing in both healthy persons and pain patients [37-41]. All participants were Dutch native speakers and 18 to 65 years old. Participants were recruited by calls on social media and through advertisements on the Ghent University website, placed in health magazines and an information brochure of an association for patients with whiplash. Furthermore, informative flyers and posters were distributed in different medical institutes and associations in Flanders (various hospitals, physical therapist practices, and medical physician practices).

Inclusion criteria for patients with CINP and CWAD were persistent neck pain lasting more than 3 months [42] with a mean pain intensity of more than 3 of 10 on the Numeric Rating Scale (NRS) during the preceding month. All chronic neck pain patients had to report mild/moderate to severe pain-related disability, established by a score of 10 or more of a maximum of 50 on the Neck Disability Index [43]. Additionally, chronic neck pain patients had to report stability of pain medication intake for at least 4 weeks before study participation.

A specific inclusion criterion for patients with CINP was persistent idiopathic (non-traumatic) neck pain. Patients with CINP were excluded if they ever experienced a whiplash trauma, or any other specific causes of neck pain, e.g. cervical hernia with clinical symptoms.

Patients with CWAD were included only if they had neck pain resulting from a motor vehicle crash or traumatic event and classifiable as WAD II A, B, or C on the modified [44] Quebec Task Force Scale [45]. Patients with CWAD grades I, III (neurological signs) or IV (fracture or dislocation) on the modified Quebec Task Force Scale were excluded. Additionally, CWAD patients who lost consciousness as a result of the motor vehicle crash or traumatic event, and patients who had suffered posttraumatic amnesia were excluded [46].

Healthy pain-free women could participate only if they were pain-free on each test day (NRS score of <2/10); had no history of neck-shoulder-arm pain for more than 8 consecutive days during the preceding year, with a pain intensity of 2 or more on the NRS; no medical consultation for neck-shoulder-arm pain during the preceding year; and no history of

whiplash trauma. Additionally, healthy controls were included only if they had a score of less than 8 of 50 on the Neck Disability Index.

General exclusion criteria for all study groups were the presence of major depression, anxiety, psychiatric, neurologic, metabolic, cardiovascular, and inflammatory disorders, fibromyalgia, chronic fatigue syndrome, and a history of neck or shoulder girdle surgery. Furthermore, all participants completed the MRI safety checklist and participants who presented contraindications for MRI were excluded. To preclude confounding factors, all participants were asked to discontinue intake of non-opioid analgesics 48 hours before study participation. Continuation of intake of narcotic analgesics was allowed and medication use of each participant was questioned in detail. In addition, participants were asked to avoid heavy physical activities, and to refrain from consuming alcohol, caffeine, and nicotine on the day of testing. Finally, brain microhemorrhages related to a traumatic event were excluded based on visual inspection of T2*-weighted brain images.

Self-reported pain and disability measures

On each test day, participants scored current neck pain intensity on an 11-point verbal numeric rating scale (VNRS-11). Scores range from 0 to 10, with 0 reflecting 'no pain at all' and 10 reflecting 'the worst pain imaginable'. In addition, patients reported the frequency of neck pain complaints in number of days per week. The Dutch Neck Disability Index was used to investigate self-reported pain-related disability levels (0-50) [43, 47]. Higher scores on the Neck Disability Index indicate higher levels of pain-related disability. The Dutch language version of the Neck Disability Index has been proven to be reliable and valid to assess self-reported disability in patients with chronic neck pain [48-51].

Cognitive performance

Subjective cognitive performance

Participants completed the Dutch modified Perceived Deficits Questionnaire (mPDQ) to investigate subjective cognitive performance (0-72). This questionnaire investigates self-perceived cognitive problems in 4 different cognitive subdomains, i.e. prospective memory, retrospective memory, attention and concentration, and organization and planning, during the preceding 4 weeks. Symptoms are rated on a 5-point Likert scale from never (0) to almost always (4). Higher scores represent more self-perceived cognitive deficits. The validity and reliability of the English mPDQ have been demonstrated in patients with CWAD and healthy persons [52].

Objective cognitive performance

The Trail Making Test (TMT) was administered in order to objectively obtain an instrumented measure of cognitive performance [53]. This test consists of two parts, trail A and trail B. The TMT part A requires mainly visuoperceptual and processing speed abilities, whereas TMT part B reflects working memory and task-switching ability. In trail A, the participant was instructed to draw lines connecting 25 numbers in ascending order as fast as possible, without lifting the pencil from the page. In trail B, the participant had to draw lines alternating between numbers and letters in ascending order (going from 1 to A, from A to 2, etc.). The goal of the TMT was to finish part A and part B as quickly and as accurate as possible. The researcher explained each part and participants completed a practice version containing fewer items. The time taken to complete each part of the test, and a switch cost, calculated by subtracting completion time of part A from part B, were used as outcome measures. The TMT (B-A) difference minimizes visuoperceptual and working memory demands, thus providing an indication of executive function [53]. Higher scores on completion time and switching cost denote worse cognitive performance. The TMT has been demonstrated to be valid for assessing cognitive deficits [53].

Self-reported and experimental measures of pain processing

Pain catastrophizing

The Dutch Pain Catastrophizing Scale (PCS) (0-52) was used to evaluate three components of catastrophizing: rumination, magnification, and helplessness [54]. Higher scores represent higher levels of pain catastrophizing. The Dutch PCS has sufficient test-retest reliability [55, 56] and the factor structure is confirmed in chronic pain patients and healthy individuals [57].

Pain hypervigilance

The Dutch Pain Vigilance and Awareness Questionnaire (PVAQ) was administered to assess the level of vigilance towards pain (0-80). Higher scores indicate a higher degree of pain vigilance and awareness. The PVAQ has been shown to be valid and reliable to measure pain vigilance in healthy individuals [58] and chronic pain patients [59].

Self-reported symptoms of central sensitization

All participants completed the Dutch language version of the Central Sensitization Inventory (CSI). The CSI is a self-report screening instrument for the measurement of clinical symptoms of CS (0-100) in chronic pain populations [60, 61]. Higher CSI scores denote a higher degree of CS symptoms. The Dutch CSI has been shown to have good internal consistency, excellent test-retest reliability, and good discriminative power to differentiate between healthy persons and chronic pain patients [60]. Neblett et al [62] determined that a CSI score of 40 of 100 best distinguished between a group of central sensitivity syndrome patients (CSI scores ≥40/100) and a group of non-central sensitivity syndrome patients (sensitivity = 81%, specificity = 75%).

Local and distant hyperalgesia

The PPTs were measured unilaterally with a digital pressure algometer with a 1 cm^2 tip (Wagner Instruments, FDX, Greenwich, Connecticut), both at a symptomatic local region (middle trapezius muscle midway between the spinous process of C7 and the lateral

border of the acromion) to evaluate local hyperalgesia and at a distant asymptomatic region (quadriceps muscle midway between the anterior superior iliac spine and the basis patellae) to evaluate widespread or distant hyperalgesia [63, 64]. The PPTs were assessed on the more painful side [65]. In healthy women and when patients experienced the same amount of neck pain on both sides, PPTs were tested on the dominant handedness side. The PPTs were assessed in a randomized order (with Research Randomizer, https://www.randomizer.org). During the test procedure, participants were seated and pressure was gradually increased at a rate of 1 kgf/s until the participants reported the first sensation of unpleasantness. The PPT was determined as the mean of 2 consecutive (30 seconds in between) measurements. Decreased PPTs in the patient groups compared to healthy controls at the middle trapezius muscle indicate local hyperalgesia, whereas decreased PPTs at the quadriceps muscle indicate distant hyperalgesia. This technique has been found to be reliable [66]. In addition, the intratester reliability of PPT measurements has been reported to be satisfactory to good (intraclass correlation coefficient= 0.78-0.93) [67].

Efficacy of Conditioned Pain Modulation (CPM)

The presence of dysfunctional endogenous pain inhibition was investigated by evaluating the efficacy of CPM by applying a CPM paradigm. This paradigm relies on the "pain-inhibits-pain" mechanism, in which one noxious stimulus is used as a conditioning stimulus to induce a reduction in the perception of pain from another test stimulus [68]. The conditioning stimulus for eliciting CPM was the cold pressor test. The assessment of PPTs was used as the test stimulus. For the conditioning stimulus, the contralateral hand (of the PPT side) [69] was first immersed in water maintained at room temperature (22°C) for 1 minute to standardize the hand temperature [70], before immersing this hand (up to the wrist) in a refrigerated bath (VersaCool, Thermo Fisher Scientific, Newington NH, USA) with circulating cold water maintained at 12±1°C [71]. Participants were asked to keep their hand in the water bath for 2 minutes [70]. Meanwhile, the PPT was re-evaluated at the quadriceps muscle, 45 seconds after immersing the hand (again twice with an interval of 30 seconds) [72]. If participants removed the hand from the water before the end of the 2 minutes, the measurement was registered as missing. For analysis of CPM efficacy, the mean PPT measured before the cold pressor test was subtracted from the mean PPT measured during the cold pressor test. Hence, a lower CPM value reflected less efficient endogenous pain inhibition. The intrasession and intraclass correlation coefficients for the cold pressor test have been shown to be excellent (0.85) [72].

MRI data acquisition

Magnetic Resonance images were acquired on a 3T Siemens Magnetom TrioTim MRI scanner (Siemens, Erlangen, Germany) equipped with a 32-channel matrix head coil, at the Ghent University Hospital. High-resolution T1-weighted images of the brain were acquired using a three-dimensional magnetization prepared rapid acquisition gradient echo (MP-RAGE) (repetition time [TR] = 2250 ms, echo time [TE]= 4.18 ms, voxel size= 1 x 1 x 1 mm^3, FoV= 256 mm, flip angle= 9°, 176 slices, 1mm slice thickness, acquisition time= 5'14''). All T1-weighted anatomical scans were visually checked for overall quality and motion artefacts.

In addition, axial T2*-weighted brain images were acquired using a T2*-weighted acquisition gradient echo with TR= 839 ms, TE= 18.60 ms, voxel size= 1 x 0.7 x 3 mm^3, FoV= 230 mm, flip angle= 20°, 3 mm slice thickness, and acquisition time of 3' 48''. All T2*-weighted images were visually inspected by 2 expert neuroradiologists (KD, EG) to evaluate and exclude possible microhemorrhages related to a traumatic event.

MRI data processing

The high-resolution T1-weighted anatomical scans were analyzed utilizing the FreeSurfer v5.3.0 software package, which is documented and freely available (http://surfer.nmr.mgh.harvard.edu). The analyses were performed utilizing additional computing resources from the high performance computing (HPC) TIER1 cluster at the University of Ghent (http://www.ugent.be/hpc/). The FreeSurfer analysis suite was used to extract cortical and subcortical GM volumes using an automated approach described in detail in prior publications (for an overview see Fischl 2012 [73]). Previous research has shown that this automated procedure yields accurate and reliable results [74]. Briefly, image processing included (1) removal of non-brain tissue using a hybrid watershed/surface deformation procedure (skull stripping) [75], (2) automated Talairach transformations, (3) segmentation of the subcortical white matter and deep GM volumetric structures [74, 76], (4) intensity normalization (77), (5) tessellation of the boundary between GM and white matter, automated topology correction [78, 79] and (6) surface deformation along intensity gradients for optimal placement of the borders between GM, white matter and cerebrospinal fluid [80-82]. Automated parcellation of the cerebral cortex into units with respect to gyral and sulcul structures was performed within each hemisphere using the Desikan atlas [83]. Furthermore, an automated segmentation (Aseg) of subcortical GM regions within each hemisphere was performed in FreeSurfer [74, 76]. Also, an estimate of total intracranial volume was obtained for each subject.

Two independent researchers (IC, RDP) visually checked the data quality of the FreeSurfer processing output including the accuracy of skull stripping, registration, segmentation, and cortical surface reconstruction. Poor data quality, such as inclusion of dura in the pial surface after skull stripping, and surface deformations, was revealed in 12 participants (healthy controls =3, CINP =3, CWAD =6). These GM volume datasets were excluded from all further analyses. All other data was of good quality and was used for further analyses.

Regions of interest

Grey matter volume was extracted from regions of interest (ROIs). Cortical and subcortical regions, which have been reported to be involved in processing pain and cognition in previous studies, were selected as ROIs. Furthermore, ROIs were defined based on observations from previous studies in patients with chronic pain regarding GMV alterations [15, 19, 20, 84], and regarding relationships between GMV alterations, and measures of cognition and pain [15, 85-87]. The ROIs constituting pain and cognitive processing regions included two subcortical GM structures: amygdala and thalamus (see **Fig. 1** for subcortical ROIs), and 12 cortical regions selected from the Desikan atlas [83]: caudal anterior cingulate, rostral anterior cingulate, posterior cingulate, rostral middle frontal, medial orbitofrontal, lateral orbitofrontal, superior parietal, insula, postcentral, precuneus, pars orbitalis, and supramarginal cortex (see **Fig. 1** for cortical ROIs). For each ROI, GMV was calculated for the right and left hemisphere separately. In addition, the volumes of total subcortical GM and total cortical GM were obtained.

Statistical analyses

All statistical analyses were performed with IBM SPSS Statistics 22.0 (IBM SPSS, Armonk, New York). First, the normality of variables was checked with the Shapiro-Wilk test and by visual evaluation of quantile-quantile plots and histograms. Additionally, the equality of variance was examined with the Levene's test. Only normally distributed data with an equality of variance were analyzed with parametric tests. Otherwise, non-parametric tests were applied.

The comparability of study groups for age, current neck pain intensity, pain duration, and other demographics was explored with a one-way ANOVA with post-hoc pairwise comparisons using Bonferroni correction (*Family Wise Error Rate (FWER)* <0.05), or with the Kruskal-Wallis test with post-hoc pairwise comparisons using the Mann-Whitney *U* test. Differences measured with the Mann-Whitney *U* test were assumed to be significant only below the 0.017 (Bonferroni correction: 0.05/3) level. Categorical data were analyzed with the Fisher's exact test.

Subsequently, differences between study groups regarding cognitive performance and pain processing were explored using one-way ANOVA (post-hoc pairwise comparisons using Bonferroni correction, FWER <0.05) or the Kruskal-Wallis test (post-hoc pairwise comparisons using the Mann-Whitney *U* test, $p<0.017$). An analysis of covariance (ANCOVA) model, controlling for the potentially confounding factor of age, was used to determine significant group differences in GMV of the selected ROIs, and total subcortical and cortical GMV (post-hoc pairwise comparisons using Bonferroni correction, FWER <0.05).

Finally, correlations among measures of cognition and pain on one hand, and regional GMV on the other hand in both chronic neck pain conditions were investigated with group-specific Spearman correlation analyses. To correct for multiple comparisons, we deemed only Spearman correlations below the 0.01 level (2-tailed) to be significant.

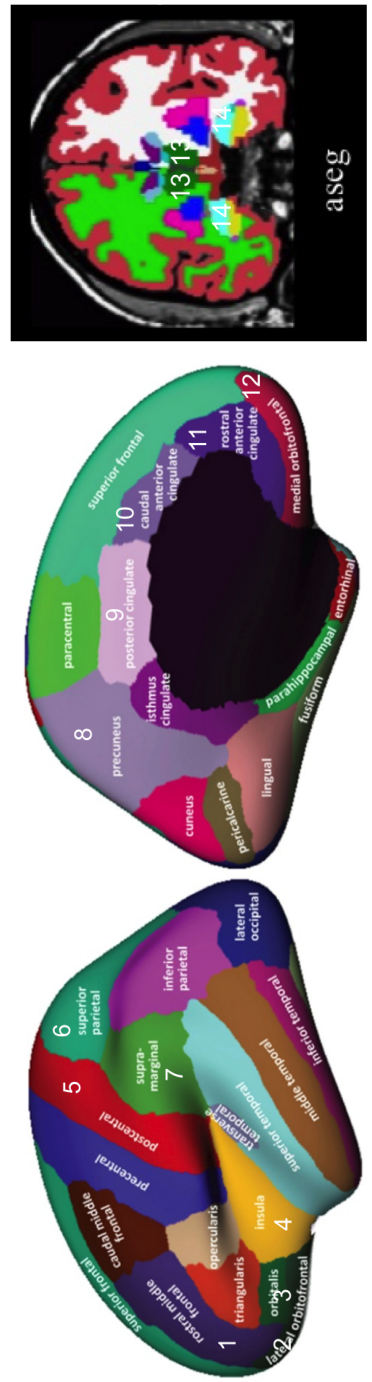


Figure 1 Lateral (left fig.) and medial (centre fig.) view of the cortical parcellation of the Desikan atlas [1] displayed on an inflated template (https://surfer.nmr.mgh.harvard.edu). Numbered regions indicate the cortical regions of interest: 1) Rostral middle frontal; 2) Lateral orbitofrontal; 3) Pars orbitalis; 4) Insula; 5) Postcentral; 6) Superior parietal; 7) Supramarginal; 8) Precuneus; 9) Posterior cingulate; 10) Caudal anterior cingulate; 11) rostral anterior cingulate; 12) medial orbitofrontal. **View (right fig.) of the subcortical parcellation of the Aseg atlas** (2) (https://surfer.nmr.mgh.harvard.edu). Numbered regions indicate the subcortical regions of interest: 13) Thalamus; 14) Amygdala.

Correlation coefficients were deemed low between 0.30 to 0.50, moderate between 0.50 to 0.70, high between 0.70 to 0.90, and very high between 0.90 to 1.00 [88].

Results

Differences between patients with idiopathic and traumatic chronic neck pain compared to healthy controls

Demographic characteristics and self-reported pain and disability measures

The results of demographic characteristics and self-reported pain and disability measures of 81 female participants (25 healthy controls, 31 patients with CINP, 25 patients with CWAD) are shown in **Table 1**. All study groups were comparable in age, body height, body weight, body mass index, education level, smoking status, menstrual phase, and handedness (p>0.05). Furthermore, both groups with chronic neck pain were comparable in medication use, neck pain duration, and frequency of neck pain complaints per week (p>0.05). Participants with CWAD reported significantly higher current neck pain intensity on the clinical and MRI test day, and significantly more pain-related disability than participants with CINP (p<0.01).

Ninety-one percent of all participants were right-handed. This is a representative sample regarding handedness because approximately 10 percent of the general population is ambidextrous or left-handed [89]. The ANCOVA with age as covariate and handedness as fixed-factor, revealed no significant main effect of handedness on total and regional GMV. Therefore, the GMV results of the left- and right-handed women were analyzed together.

Cognitive performance

Subjective cognitive performance

Compared with healthy controls, patients with CINP (p=0.009) and patients with CWAD (p<0.001) reported more self-perceived cognitive deficits, as presented in **Table 1**. Moreover, CWAD patients reported more self-perceived cognitive deficits compared to patients with CINP (p=0.001).

Objective cognitive performance

The time needed to perform TMT part A (p=0.002) and TMT part B (p=0.004) was significantly longer in the CWAD group compared to the healthy control group, denoting worse objective cognitive performance in patients with CWAD (**Table 1**). In addition, the time needed to perform TMT part A (p=0.003) and TMT part B (p=0.009) was significantly longer in CWAD patients compared to CINP patients. Despite the differences in completion time, no significant group differences were revealed for executive control or switching cost (TMT (B-A) difference), (p's>0.05).

Self-reported and experimental measures of pain processing

Pain catastrophizing and pain hypervigilance

As can be seen in **Table 1**, maladapted pain cognitions including pain catastrophizing and hypervigilance were significantly higher in patients with CWAD compared to healthy women (p=0.003; p=0.035, respectively). No significant differences between CINP patients and healthy controls were found regarding pain catastrophizing and pain hypervigilance (p>0.05).

Self-reported central sensitization symptoms

Both patient groups reported significantly more self-perceived CS symptoms compared to healthy pain-free women (p<0.001) (**Table 1**). Moreover, patients with CWAD experienced significantly more CS symptoms compared to patients with CINP (p=0.005).

Local and distant hyperalgesia

Decreased PPTs were demonstrated at the middle trapezius muscle and quadriceps muscle in patients with CWAD (p=0.001, p=0.008, respectively) but only at the middle trapezius muscle in patients with CINP, relative to the results for healthy women (p=0.009) (**Table 1**).

Efficacy of Conditioned Pain Modulation

The CPM value measured at the quadriceps muscle was significantly lower in patients with CWAD compared to healthy women (p=0.010), as presented in **Table 1**.

Total cortical and subcortical grey matter volume

As can be seen in **supplementary Table A**, the ANCOVA with age as covariate revealed no significant differences between all study groups for total intracranial volume (p=0.109), total cortical GMV (p=0.198), and total subcortical GMV (p=0.510). Therefore, we decided not to include these metrics in further analyses.

Regional based grey matter volume

The significant results of the ANCOVA with age as covariate investigating differences in GMV of pain and cognitive processing regions between patients with CINP and CWAD, and healthy controls are presented in **Figure 2** and supplementary **Table A**. The non-significant ANCOVA results for GMV of the ROIs are shown in supplementary **Table B**.

The ANCOVA revealed decreased GMV in the left posterior cingulate cortex (p=0.047), the right lateral orbitofrontal cortex (p=0.023), and the left supramarginal cortex (p=0.012) in patients with CWAD compared to healthy controls (Bonferroni-adjusted p-values). Furthermore, decreased GMV in the left posterior cingulate cortex (p=0.035), and the right superior parietal cortex (p=0.008) in CWAD patients compared to CINP patients was demonstrated with the ANCOVA (Bonferroni-adjusted p-values). No significant differences

Table 1 Demographic characteristics, self-reported pain and disability measures, maladaptive pain cognitions, self-reported symptoms of central sensitization, subjective and objective cognitive performance, local and distant hyperalgesia and conditioned pain modulation efficacy.

			Mean	Median	SD	Range (min-max)	IQR	Test statistic (*P*-value)	*P*-value post-hoc
Demographic characteristics	**Age (y)**[a]	***HCON***	30.32	24.00	13.20	18.00 – 62.00	22.50 – 36.50	5.393 (0.067)	NA
		CINP	34.93	34.00	10.85	18.00 – 54.00	26.00 - 45.00		
		CWAD	35.32	35.00	10.83	21.00 – 58.00	25.00 - 43.50		
	Body height (cm)[b]	***HCON***	167.16	167.00	6.01	155.00 – 178.00	163.00 – 170.00	0.044 (0.957)	NA
		CINP	166.76	168.00	5.28	157.00 – 175.00	163.00 – 170.50		
		CWAD	167.12	166.00	5.38	155.00 – 176.00	163.50 – 172.00		
	Body weight (kg)[a]	***HCON***	60.87	59.00	7.29	51.00 – 81.00	55.35 - 65.00	1.500 (0.472)	NA
		CINP	63.38	60.50	9.02	50.00 – 86.00	56.75 – 69.25		
		CWAD	62.02	60.00	12.67	48.00 – 95.00	51.00 – 67.50		
	Body mass Index (kg/m²)[a, †]	***HCON***	21.76	21.80	2.07	18.07 – 26.75	20.45 – 23.06	1.742 (0.418)	NA
		CINP	22.64	22.74	2.68	18.65 – 29.07	20.31 – 24.45		
		CWAD	22.17	21.14	4.18	16.65 – 32.05	19.14 – 23.59		
			Frequencies						
	Education level n (%)[c] No degree; lower secon.; Higher second.; Higher edu.	***HCON***	0 (0); 1 (4); 6 (24); 18 (72)					0.782 (0.991)	NA
		CINP	0 (0); 2 (6.5); 7 (22.6); 20 (64.5)						
		CWAD	0 (0); 1 (4); 5 (20); 19 (76)						
	Smoker n (%)[c] Smoker; former smoker; non-smoker	***HCON***	1 (4); 3 (12); 21 (84)					4.801 (0.299)	NA
		CINP	1 (3.2); 9 (29); 18 (58.1)						
		CWAD	3 (12); 6 (24); 16 (64)						
	Menstrual phase clinical test day n (%)[c] Follicular phase (day 1 to	***HCON***	14 (56); 2 (8); 4 (16); 1 (4); 2 (8); 1 (4)					10.374 (0.344)	NA
		CINP	16 (51.6); 1 (3.2); 6 (19.4); 1 (3.2); 4 (12.9); 1 (3.2)						
		CWAD	8 (33.3); 0 (0); 9 (37.5); 0 (0); 3 (12.5); 4 (16.7)						
	Handedness n (%)[c] (LH; RH)	***HCON***	2 (8) ; 23 (92)					0.691 (0.884)	NA
		CINP	2 (6.5); 29 (93.5)						
		CWAD	3 (12) ; 22 (88)						
***Demographic characteristics*: medication use**	**Analgesics – antipyretics n (%)**[c]	***HCON***	0 (0)					2.970 (0.158)	NA
		CINP	3 (9.7)						
		CWAD	7 (28)						
	Narcotic analgesics n (%)[c]	***HCON***	0 (0)					1.222 (0.455)	NA
		CINP	0 (0)						
		CWAD	1 (4)						
	Benzodiazepines n (%)[c]	***HCON***	0 (0)					3.897 (0.082)	NA
		CINP	1 (3.20)						
		CWAD	5 (20)						
	Antidepressants (%)[c]	***HCON***	0 (0)					0.849 (1.000)	NA
		CINP	3 (9.70)						
		CWAD	1 (4)						
			Mean	**Median**	**SD**	**Range (min-max)**	**IQR**	**Test statistic (*P*-value)**	***P*-value post-hoc**
Self-reported pain measures	**Neck pain duration (months)**[a]	***HCON***	NA	NA	NA	NA	NA	0.076 (0.783)	NA
		CINP	92.96	60.00	88.21	4.00 – 300.00	24.00 – 138.00		
		CWAD	86.87	51.50	96.13	6.00 – 440.00	26.25 – 115.00		
	Days/week neck pain?[a]	***HCON***	NA	NA	NA	NA	NA	3.048 (0.081)	NA
		CINP	5.14	5.00	1.61	3.00 – 7.00	4.00 – 7.00		
		CWAD	5.95	7.00	1.70	2.00 – 7.00	5.00 – 7.00		
	Current neck pain intensity (VNRS/10)_C[a, †]	***HCON***	0.08	0.08	0.28	0.00 – 1.00	-0.03 - 0.19	44.391 (**< 0.001**)	**< 0.001**[d] **< 0.001**[e] **< 0.011**[f]
		CINP	3.85	3.85	2.57	0.00 – 8.00	2.91 - 4.80		
		CWAD	5.76	5.76	2.65	0.00 – 10.00	4.67 - 6.85		
	Current neck pain intensity (VNRS/10)_M[a, †]	***HCON***	0.00	0.00	0.00	0.00 – 0.00	0.00 – 0.00	72.467 (**< 0.001**)	**< 0.001**[d] **< 0.001**[e] **< 0.001**[f]
		CINP	3.43	3.43	1.98	0.00 – 7.00	2.71 – 4.16		
		CWAD	5.98	5.98	2.28	1.00 – 10.00	5.04 – 6.92		
Self-reported disability	**Neck Disability Index (/50)**[a, †]	***HCON***	2.76	2.00	1.61	1.00 – 6.00	1.00 – 4.00	54.439 (**< 0.001**)	**< 0.001**[d] **< 0.001**[e] **0.001**[f]
		CINP	16.36	16.00	5.03	10.00 – 27.00	12.00 - 20.50		
		CWAD	23.04	23.00	6.93	10.00 – 37.00	18.00 - 27.50		

Table 1 Continued.

			Mean	Median	SD	Range (min-max)	IQR	Test statistic (*P*-value)	*P*-value post-hoc
Self-reported disability	**Neck Disability Index (/50)**[a, †]	***HCON***	2.76	2.00	1.61	1.00 – 6.00	1.00 – 4.00	54.439 **(< 0.001)**	**< 0.001**[d] **< 0.001**[e] **0.001**[f]
		CINP	16.36	16.00	5.03	10.00 – 27.00	12.00 - 20.50		
		CWAD	23.04	23.00	6.93	10.00 – 37.00	18.00 - 27.50		
Subjective cognitive performance	**mPDQ Total (/72)**[a, †]	***HCON***	11.52	10.00	7.00	1.00 – 25.00	6.00 – 16.00	26.448 **(< 0.001)**	**0.009**[d] **< 0.001**[e] **0.001**[f]
		CINP	18.85	14.00	10.34	5.00 – 44.00	11.00 – 22.00		
		CWAD	31.83	28.50	14.61	6.00 – 57.00	19.00 – 46.50		
Objective cognitive performance (TMT)	**TMT part A (sec)**[a, †]	***HCON***	19.11	18.76	3.83	12.28 - 29.75	16.22 - 21.83	12.757 **(0.002)**	0.586[d] **0.002**[e] **0.003**[f]
		CINP	19.80	19.37	4.29	11.56 - 30.13	16.86 - 22.41		
		CWAD	29.00	27.09	14.27	15.06 - 81.00	18.95 - 31.82		
	TMT part B (sec)[a, †]	***HCON***	41.86	34.37	24.02	21.44 – 128.00	27.86 – 45.89	10.747 **(0.005)**	0.317[d] **0.004**[e] **0.009**[f]
		CINP	42.73	37.00	23.38	26.6 – 148.00	31.05 – 44.36		
		CWAD	66.02	44.83	48.62	27.93 – 251.00	37.13 – 79.50		
	TMT (B-A)[a, †]	***HCON***	22.75	16.46	21.60	2.25 – 98.25	11.64 – 24.61	2.333 (0.311)	NA
		CINP	22.93	17.83	21.09	7.08 – 121.02	13.78 – 24.07		
		CWAD	37.02	20.93	37.83	5.85 – 170.00	13.28 – 57.65		
Maladaptive pain cognitions	**Pain Catastrophizing (/52)**[b]	***HCON***	9.76	10.00	8.61	0.00 – 30.00	1.00 – 18.00	9.740 **(0.004)**	0.308[d] **0.003**[e] 0.166[f]
		CINP	13.65	13.00	7.19	1.00 – 26.00	6.00 - 19.50		
		CWAD	18.24	19.00	10.09	0.00 – 37.00	10.00 - 27.50		
	Pain Hypervigilance (/80)[b]	***HCON***	30.24	32.00	10.88	10.00 – 55.00	20.50 – 39.00	6.560 **(0.026)**	0.096[d] **0.035**[e] 1.000[f]
		CINP	36.97	37.00	12.36	16.00 – 70.00	29.50 – 46.00		
		CWAD	38.48	38.00	10.28	16.00 – 56.00	30.00 - 46.50		
Self-reported symptoms of central sensitization	**Central sensitization inventory (/100)**[a, †]	***HCON***	20.25	20.00	6.42	9.00 – 35.00	16.00 – 23.00	44.731 **(< 0.001)**	**< 0.001**[d] **< 0.001**[e] **0.005**[f]
		CINP	40.48	40.00	10.02	22.00 – 68.00	35.00 – 47.50		
		CWAD	49.33	48.50	13.82	13.00 – 67.00	41.00 – 63.25		
Local HA	**PPT trapezius (kgf)**[a]	***HCON***	4.42	3.69	1.90	1.86 – 9.81	3.27 – 5.75	12.295 **(0.002)**	**0.009**[d] **0.001**[e] 0.299[f]
		CINP	3.24	2.76	1.69	1.18 – 7.43	2.01 – 4.04		
		CWAD	2.81	2.46	2.01	0.13 – 9.30	1.68 – 3.41		
Distant HA	**PPT quadriceps (kgf)**[b]	***HCON***	4.95	4,38	1.57	2.94 – 8.40	3.71 – 6.16	4.768 **(0.011)**	0.262[d] **0.008**[e] 0.401[f]
		CINP	4.09	3.47	2.03	1.45 – 9.72	2.54 – 5.68		
		CWAD	3.34	3.15	1.87	0.30 – 7.72	1.95 – 4.74		
CPM efficacy	**CPM quadriceps** (PPT quadriceps during CPT minus PPT quadriceps before CPT)[b]	***HCON***	1.19	1.31	0.70	-0.14 – 3.00	0.68 – 1.51	4.978 **(0.010)**	1.000[d] **0.010**[e] 0.054[f]
		CINP	1.04	0.90	1.02	-0.59 - 3.29	0.41 – 1.66		
		CWAD	0.45	0.37	0.68	-0.75 - 1.87	-0.08 – 1.02		

The distribution of the continuous data within each group was assessed by histograms, QQ-plots, and the Shapiro-Wilk test.
[a]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and for post-hoc pairwise comparisons the Mann-Whitney U test. Shapiro-Wilk test $p<0.05$ and visual inspection of the QQ-plot and histogram within each group provided information that the data were not normally distributed. To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.017 level (Bonferonni correction: 0.05/3). [b]= Data which were assumed to be normally distributed and were variances were equally distributed across groups were analyzed with one-way ANOVA (F-test) and post-hoc pairwise comparisons were applied using Bonferroni correction ($p<0.05$). [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. [†]Variances were not equally distributed across groups, Levene's test $p<0.05$, [d]= *p*-value for significant differences between CON-CINP, [e]= *p*-value for significant differences between CON-CWAD, [f]= *p*-value for significant differences between CINP-CWAD. There were 3 missings (1 HCON, 2 CINP) for menstrual phase. Abbreviations: y= years. CON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain. VNRS= verbal numeric rating scale. SF-36= Short Form Health Survey, No degr= no degree, Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, HA= hyperalgesia, CPM= conditioned pain modulation, CPT= cold pressor test, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, kgf= kilogram force, PPT= pressure pain thresholds, VNRS= verbal numeric rating scale, IQR= interquartile range. Data of 81 subjects were analysed (25 healthy controls, 31 CINP, 25 CWAD).

in regional GMV were found between patients with CINP and healthy women (*p's*>0.05). In addition, no significant subcortical GMV differences were found in the amygdala and thalamus between all study groups (*p*>0.05).

Relationships between regional grey matter volume, and cognitive deficits, pain intensity, and pain processing in patients with idiopathic and traumatic chronic neck pain

CINP

The results of the Spearman correlation (r_s) analyses between GMV of regions involved in pain and cognitive processing, and cognitive deficits, pain intensity, and pain processing in patients with CINP are shown in **Tables 2a** and **2b**.

In the CINP group, only 4 significant correlations were revealed. A moderate relationship was found between increased severity of self-reported cognitive deficits, and decreased GMV of the left rostral anterior cingulate cortex (r_s= -.499; *p*=0.008). Furthermore, lower visuoperceptual abilities were moderately correlated with decreased GMV of the right thalamus (r_s= -0.529; *p*=0.003). Also, decreased task-switching capacity was moderately correlated with decreased GMV of the left medial orbitofrontal cortex (r_s= -.565; *p*=0.001). A moderate relationship was observed between decreased GMV of the left medial orbitofrontal cortex, and worse executive control (r_s= -.619; *p*<0.001).

No significant correlations among pain intensity, maladapted pain cognitions, CS symptoms, and experimental measures of pain processing, and regional GMV were demonstrated (*p*>0.01).

CWAD

The results of the Spearman correlation (r_s) analyses between GMV of regions involved in pain and cognitive processing, and cognitive deficits, pain intensity, and pain processing in patients with CWAD are displayed in **Tables 3a** and **3b**.

In the CWAD group, more robust correlations were found compared to the CINP group. Moderate correlations were revealed between increased severity of self-reported cognitive deficits, and decreased GMV of the left pars orbitalis (r_s= -.543; *p*=0.006), the left amygdala (r_s= -0.598; *p*=0.002), and the right medial orbitofrontal cortex (r_s= -0.548; *p*=0.006). Furthermore, decreased task-switching capacity was moderately correlated with decreased GMV of the right rostral anterior cingulate cortex (r_s= -.588; *p*=0.002), the right posterior cingulate cortex (r_s= -0.538; *p*=0.007), the left rostral middle frontal cortex (r_s= -0.604; *p*=0.002), and the left insula (r_s= -0.539; *p*=0.007). In addition, worse executive control was moderately correlated with decreased GMV of the left rostral middle frontal cortex (r_s= -0.617, *p*=0.001), the left lateral orbitofrontal cortex (r_s= -0.539, *p*=0.007), the left insula (r_s= -0.634, *p*=0.001), the right posterior cingulate cortex (r_s= -0.594, *p*=0.002), and the right lateral orbitofrontal cortex (r_s= -0.569, *p*=0.004).

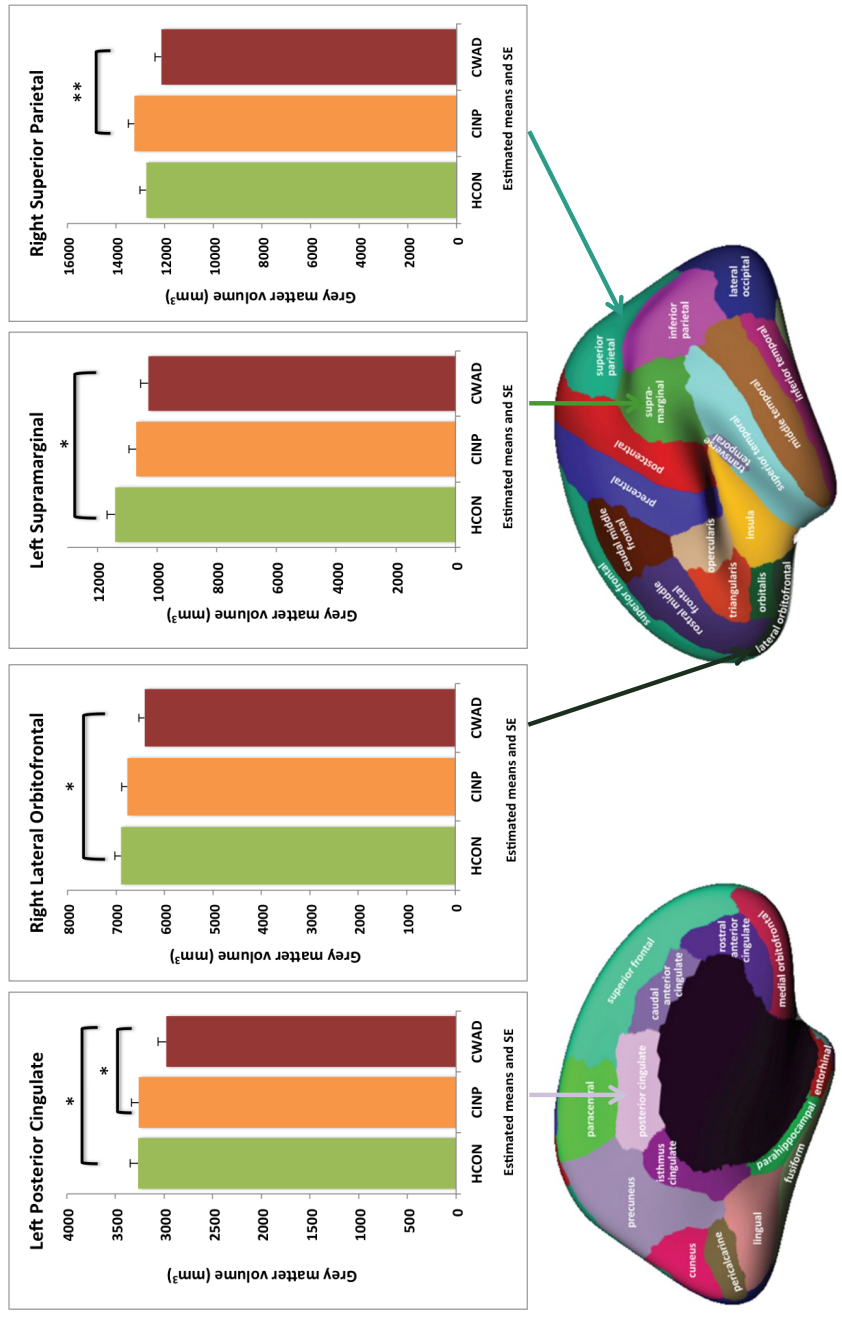


Figure 2 Regional grey matter volume decrease in patients with CWAD compared to CINP patients and healthy women. Abbreviations: HCON= healthy pain-free controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, SE= standard error. Data were analyzed using ANCOVA with age as covariate and post-hoc pairwise comparisons were applied using Bonferroni correction. *= *p*<0.05, **= *p*<0.01

Table 2a Spearman correlations between **regional cortical and subcortical grey matter volume (LH),** and **self-reported and experimental measures of pain and cognition** in patients with CINP.

	Caudal ACC	Rostral ACC	PCC	Rostral middle frontal	Medial OBF	Lateral OBF	Superior Parietal	Insula	Postcentral	Precuneus	Pars Orbitalis	Supra-marginal	Amygdala	Thalamus
CINP (n=31)														
Self-perceived cognitive performance (mPDQ)														
Total score	-0.378	**-0.499**	-0.356	-0.201	-0.084	-0.177	-0.100	-0.335	-0.171	-0.067	-0.066	-0.179	0.006	-0.017
	0.052	*0.008*	*0.068*	*0.315*	*0.678*	*0.377*	*0.619*	*0.088*	*0.394*	*0.739*	*0.745*	*0.372*	*0.978*	*0.932*
Objective cognitive performance (TMT)														
Part A	-0.229	-0.208	-0.307	-0.196	-0.293	-0.269	0.030	-0.398	-0.005	0.044	-0.105	-0.337	0.092	-0.120
	0.233	*0.279*	*0.105*	*0.309*	*0.123*	*0.158*	*0.879*	*0.033*	*0.98*	*0.819*	*0.588*	*0.073*	*0.636*	*0.536*
Part B	-0.044	-0.078	-0.334	-0.324	**-0.565**	-0.299	0.158	-0.385	-0.178	0.041	-0.290	-0.38	-0.037	-0.009
	0.821	*0.686*	*0.077*	*0.086*	*0.001*	*0.116*	*0.414*	*0.039*	*0.355*	*0.834*	*0.127*	*0.042*	*0.847*	*0.964*
B – A	-0.041	-0.088	-0.252	-0.369	**-0.619**	-0.250	0.145	-0.366	-0.335	-0.004	-0.308	-0.344	-0.083	-0.010
	0.833	*0.651*	*0.188*	*0.049*	*<0.001*	*0.190*	*0.454*	*0.051*	*0.075*	*0.983*	*0.104*	*0.068*	*0.668*	*0.961*
Self-reported pain measures														
neck pain intensity_M	-0.217	0.120	0.014	0.036	-0.066	0.181	-0.001	0.092	0.227	-0.140	0.108	-0.256	-0.042	0.293
	0.241	*0.520*	*0.939*	*0.847*	*0.723*	*0.329*	*0.994*	*0.623*	*0.219*	*0.453*	*0.564*	*0.164*	*0.823*	*0.109*
Maladaptive pain cognitions														
PCS	0.012	-0.001	-0.076	-0.051	-0.343	-0.068	-0.066	-0.122	0.227	0.056	0.039	0.166	0.274	-0.192
	0.950	*0.997*	*0.697*	*0.792*	*0.069*	*0.724*	*0.734*	*0.529*	*0.237*	*0.771*	*0.843*	*0.390*	*0.150*	*0.319*
PVAQ	-0.303	-0.246	-0.294	-0.399	-0.375	-0.227	-0.128	-0.224	-0.134	-0.252	-0.112	-0.084	0.171	-0.124
	0.110	*0.197*	*0.122*	*0.032*	*0.045*	*0.237*	*0.508*	*0.244*	*0.487*	*0.187*	*0.562*	*0.666*	*0.375*	*0.523*
Self-reported symptoms of central sensitization														
CSI	0.152	0.145	0.078	0.027	0.045	-0.002	0.044	0.124	0.113	0.104	0.036	0.249	0.001	0.150
	0.432	*0.452*	*0.688*	*0.889*	*0.816*	*0.993*	*0.819*	*0.523*	*0.559*	*0.593*	*0.853*	*0.193*	*0.996*	*0.438*
Local hyperalgesia														
PPT trapezius	-0.122	0.106	0.116	0.047	-0.091	-0.040	-0.015	0.009	-0.068	-0.310	-0.130	-0.026	-0.238	-0.127
	0.512	*0.570*	*0.533*	*0.804*	*0.627*	*0.832*	*0.936*	*0.963*	*0.717*	*0.090*	*0.484*	*0.889*	*0.198*	*0.497*
Distant hyperalgesia														
PPT quadriceps	0.090	0.225	0.088	0.060	-0.052	-0.084	0.310	0.125	0.140	-0.095	-0.074	0.004	-0.159	-0.230
	0.630	*0.224*	*0.640*	*0.750*	*0.779*	*0.653*	*0.089*	*0.501*	*0.453*	*0.610*	*0.691*	*0.981*	*0.393*	*0.214*
CPM efficacy														
CPM quadriceps	-0.057	-0.043	0.089	0.023	-0.038	0.067	-0.165	-0.072	-0.037	-0.017	-0.152	-0.001	-0.236	-0.132
	0.776	*0.832*	*0.661*	*0.911*	*0.849*	*0.738*	*0.411*	*0.721*	*0.854*	*0.934*	*0.450*	*0.998*	*0.235*	*0.512*

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons. Correlations significant at the 0.01 level (2-tailed) were deemed significant. P-values are presented below the correlation coefficient.

Abbreviations: ACC= anterior cingulate cortex, PCC= posterior cingulate cortex, OBF= orbitofrontal, CINP= chronic idiopathic neck pain, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, M= MRI test moment, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire, CSI= central sensitization inventory, PPT= pressure pain thresholds, CPM= conditioned pain modulation, LH= left hemisphere.

Table 2b Spearman correlations between **regional cortical and subcortical grey matter volume (RH),** and **self-reported and experimental measures of pain and cognition** in patients with CINP.

	Caudal ACC	Rostral ACC	PCC	Rostral middle frontal	Medial OBF	Lateral OBF	Superior Parietal	Insula	Postcentral	Precuneus	Pars Orbitalis	Supra-marginal	Amygdala	Thalamus
CINP (n=31)														
Self-perceived cognitive performance (mPDQ)														
Total score	0.151	0.151	-0.223	-0.135	-0.198	-0.188	-0.219	-0.358	-0.109	-0.218	0.168	-0.331	-0.083	-0.165
	0.453	*0.453*	*0.263*	*0.504*	*0.322*	*0.348*	*0.272*	*0.067*	*0.589*	*0.274*	*0.403*	*0.091*	*0.682*	*0.411*
Objective cognitive performance (TMT)														
Part A	0.042	-0.084	0.011	-0.230	-0.218	-0.288	-0.034	-0.285	-0.270	0.05	-0.363	-0.330	0.140	**-0.529**
	0.829	*0.664*	*0.954*	*0.231*	*0.255*	*0.130*	*0.861*	*0.134*	*0.157*	*0.796*	*0.053*	*0.081*	*0.468*	*0.003*
Part B	-0.096	-0.200	-0.061	-0.318	-0.175	-0.268	-0.158	-0.292	-0.377	0.026	0.027	-0.148	0.056	-0.314
	0.622	*0.298*	*0.753*	*0.093*	*0.363*	*0.159*	*0.414*	*0.124*	*0.044*	*0.895*	*0.888*	*0.443*	*0.772*	*0.097*
B – A	-0.088	-0.176	-0.099	-0.288	-0.210	-0.222	-0.238	-0.297	-0.379	-0.065	0.245	-0.100	-0.054	-0.182
	0.649	*0.362*	*0.611*	*0.130*	*0.273*	*0.248*	*0.214*	*0.118*	*0.042*	*0.739*	*0.201*	*0.606*	*0.782*	*0.345*
Self-reported pain measures														
neck pain intensity_M	0.145	-0.163	0.105	-0.190	0.013	0.123	0.170	0.036	0.160	-0.034	-0.075	-0.084	0.133	0.326
	0.437	*0.381*	*0.572*	*0.306*	*0.945*	*0.511*	*0.36*	*0.846*	*0.389*	*0.857*	*0.688*	*0.653*	*0.474*	*0.073*
Maladaptive pain cognitions														
PCS	0.174	0.108	0.051	-0.087	-0.156	-0.04	-0.010	-0.158	0.188	0.112	0.288	-0.069	0.109	0.022
	0.365	*0.577*	*0.793*	*0.655*	*0.420*	*0.839*	*0.958*	*0.412*	*0.329*	*0.564*	*0.129*	*0.720*	*0.574*	*0.908*
PVAQ	-0.218	-0.07	-0.138	-0.342	-0.293	-0.157	-0.177	-0.210	-0.085	-0.161	-0.009	0.236	0.022	-0.092
	0.256	*0.718*	*0.475*	*0.070*	*0.123*	*0.416*	*0.359*	*0.273*	*0.662*	*0.404*	*0.964*	*0.218*	*0.910*	*0.635*
Self-reported symptoms of central sensitization														
CSI	0.198	0.184	0.142	0.090	-0.051	0.194	-0.025	0.028	0.223	0.183	0.135	-0.031	-0.057	0.222
	0.302	*0.339*	*0.461*	*0.642*	*0.794*	*0.313*	*0.896*	*0.886*	*0.245*	*0.342*	*0.486*	*0.874*	*0.770*	*0.248*
Local hyperalgesia														
PPT trapezius	0.001	-0.185	0.255	-0.104	-0.037	0.061	0.283	0.030	-0.111	-0.273	-0.180	-0.207	-0.182	0.132
	0.995	*0.320*	*0.165*	*0.578*	*0.844*	*0.743*	*0.123*	*0.873*	*0.552*	*0.137*	*0.332*	*0.263*	*0.327*	*0.477*
Distant hyperalgesia														
PPT quadriceps	0.108	-0.045	0.238	-0.089	0.045	0.022	0.439	0.113	0.027	-0.007	-0.042	-0.052	0.000	-0.032
	0.563	*0.812*	*0.197*	*0.635*	*0.809*	*0.906*	*0.013*	*0.544*	*0.885*	*0.971*	*0.824*	*0.781*	*0.998*	*0.865*
CPM efficacy														
CPM quadriceps	-0.053	0.013	0.220	-0.027	-0.007	0.008	0.092	-0.142	-0.292	-0.07	-0.324	-0.229	-0.136	-0.030
	0.795	*0.949*	*0.271*	*0.892*	*0.971*	*0.969*	*0.647*	*0.481*	*0.140*	*0.730*	*0.099*	*0.251*	*0.500*	*0.882*

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons. Correlations significant at the 0.01 level (2-tailed) were deemed significant. P-values are presented below the correlation coefficient.

Abbreviations: ACC= anterior cingulate cortex, PCC= posterior cingulate cortex, OBF= orbitofrontal, CINP= chronic idiopathic neck pain, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, M= MRI test moment, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire, CSI= central sensitization inventory, PPT= pressure pain thresholds, CPM= conditioned pain modulation, RH= right hemispere.

Table 3a Spearman correlations between **regional cortical and subcortical grey matter volume (LH),** and **self-reported and experimental measures of pain and cognition** in patients with CWAD.

	Caudal ACC	Rostral ACC	PCC	Rostral middle frontal	Medial OBF	Lateral OBF	Superior Parietal	Insula	Postcentral	Precuneus	Pars Orbitalis	Supra-marginal	Amygdala	Thalamus
CWAD (n=25)														
Self-perceived cognitive performance (mPDQ)														
Total score	-0.154	-0.158	-0.389	-0.394	-0.170	-0.324	-0.375	-0.235	-0.427	-0.462	**-0.543**	0.078	**-0.598**	-0.355
	0.474	*0.460*	*0.060*	*0.057*	*0.428*	*0.123*	*0.071*	*0.268*	*0.037*	*0.023*	*0.006*	*0.718*	*0.002*	*0.089*
Objective cognitive performance (TMT)														
Part A	-0.122	-0.214	-0.326	-0.243	-0.016	-0.016	0.014	-0.151	-0.333	-0.129	-0.218	0.350	-0.445	0.065
	0.569	*0.314*	*0.120*	*0.252*	*0.942*	*0.941*	*0.947*	*0.482*	*0.112*	*0.549*	*0.305*	*0.094*	*0.029*	*0.764*
Part B	-0.145	-0.326	-0.505	**-0.604**	-0.314	-0.446	-0.365	**-0.539**	-0.155	-0.354	-0.498	-0.197	-0.513	-0.358
	0.498	*0.120*	*0.012*	*0.002*	*0.135*	*0.029*	*0.080*	*0.007*	*0.470*	*0.089*	*0.013*	*0.355*	*0.010*	*0.086*
B – A	-0.063	-0.158	-0.398	**-0.617**	-0.374	**-0.539**	-0.349	**-0.634**	0.041	-0.341	-0.451	-0.343	-0.417	-0.457
	0.771	*0.460*	*0.054*	*0.001*	*0.072*	*0.007*	*0.095*	*0.001*	*0.850*	*0.103*	*0.027*	*0.100*	*0.043*	*0.025*
Self-reported pain measures														
neck pain intensity_M	-0.410	-0.214	-0.193	-0.263	0.024	-0.426	-0.216	-0.179	-0.057	-0.484	-0.387	-0.086	-0.335	-0.360
	0.042	*0.305*	*0.356*	*0.204*	*0.908*	*0.034*	*0.301*	*0.393*	*0.786*	*0.014*	*0.056*	*0.683*	*0.101*	*0.078*
Maladaptive pain cognitions														
PCS	0.150	0.021	-0.016	-0.389	-0.228	-0.450	-0.207	-0.373	-0.291	**-0.522**	**-0.56**	-0.010	-0.252	-0.361
	0.474	*0.92*	*0.939*	*0.054*	*0.274*	*0.024*	*0.320*	*0.066*	*0.158*	*0.007*	*0.004*	*0.961*	*0.224*	*0.076*
PVAQ	-0.169	-0.336	-0.458	**-0.576**	-0.23	-0.365	-0.167	-0.358	-0.204	-0.293	-0.457	-0.254	-0.303	**-0.572**
	0.419	*0.100*	*0.021*	*0.003*	*0.268*	*0.073*	*0.426*	*0.079*	*0.327*	*0.155*	*0.022*	*0.221*	*0.141*	*0.003*
Self-reported symptoms of central sensitization														
CSI	0.054	-0.135	-0.389	-0.455	-0.143	-0.284	-0.261	-0.081	-0.351	-0.324	-0.491	0.16	**-0.636**	-0.333
	0.802	*0.529*	*0.06*	*0.026*	*0.506*	*0.179*	*0.218*	*0.708*	*0.093*	*0.123*	*0.015*	*0.455*	*0.001*	*0.112*
Local hyperalgesia														
PPT trapezius	0.165	0.222	0.317	0.304	0.015	0.209	0.257	0.115	**0.551**	0.354	0.482	-0.079	0.302	0.402
	0.429	*0.287*	*0.123*	*0.14*	*0.942*	*0.317*	*0.215*	*0.583*	*0.004*	*0.083*	*0.015*	*0.707*	*0.143*	*0.047*
Distant hyperalgesia														
PPT quadriceps	0.208	0.234	0.318	0.162	0.002	0.095	0.125	-0.043	0.200	0.156	0.240	-0.074	0.370	0.306
	0.317	*0.261*	*0.121*	*0.440*	*0.994*	*0.65*	*0.553*	*0.838*	*0.338*	*0.456*	*0.248*	*0.726*	*0.069*	*0.137*
CPM efficacy														
CPM quadriceps	0.064	-0.099	0.029	0.278	-0.157	-0.077	0.195	-0.186	0.468	0.372	0.024	-0.079	0.311	0.264
	0.782	*0.668*	*0.902*	*0.222*	*0.498*	*0.741*	*0.397*	*0.420*	*0.033*	*0.097*	*0.918*	*0.733*	*0.170*	*0.248*

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons. Correlations significant at the 0.01 level (2-tailed) were deemed significant. P-values are presented below the correlation coefficient.

Abbreviations: ACC= anterior cingulate cortex, PCC= posterior cingulate cortex, OBF= orbitofrontal, CWAD= chronic whiplash associated disorders, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, M= MRI test moment, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire, CSI= central sensitization inventory, PPT= pressure pain thresholds, CPM= conditioned pain modulation, LH= left hemisphere.

Table 3b Spearman correlations between **regional cortical and subcortical grey matter volume (RH),** and **self-reported and experimental measures of pain and cognition** in patients with CWAD.

	Caudal ACC	Rostral ACC	PCC	Rostral middle frontal	Medial OBF	Lateral OBF	Superior Parietal	Insula	Postcentral	Precuneus	Pars Orbitalis	Supra-marginal	Amygdala	Thalamus
CWAD (n=25)														
Self-perceived cognitive performance (mPDQ)														
Total score	-0.186	-0.329	-0.277	-0.171	**-0.548**	-0.091	-0.271	-0.401	-0.402	-0.452	-0.272	-0.303	-0.351	-0.072
	0.384	*0.117*	*0.191*	*0.426*	*0.006*	*0.673*	*0.201*	*0.052*	*0.051*	*0.026*	*0.198*	*0.150*	*0.093*	*0.737*
Objective cognitive performance (TMT)														
Part A	-0.190	-0.354	-0.115	-0.014	-0.242	0.063	-0.038	-0.144	-0.069	-0.160	-0.272	0.092	-0.216	0.275
	0.375	*0.090*	*0.592*	*0.949*	*0.255*	*0.770*	*0.861*	*0.502*	*0.748*	*0.455*	*0.198*	*0.668*	*0.311*	*0.193*
Part B	-0.262	**-0.588**	**-0.538**	-0.319	-0.485	-0.421	-0.364	-0.284	-0.299	-0.477	-0.419	-0.174	-0.356	-0.168
	0.216	*0.002*	*0.007*	*0.128*	*0.016*	*0.041*	*0.08*	*0.179*	*0.156*	*0.018*	*0.042*	*0.415*	*0.088*	*0.433*
B – A	-0.227	-0.495	**-0.594**	-0.342	-0.489	**-0.569**	-0.241	-0.312	-0.194	-0.45	-0.432	-0.213	-0.373	-0.302
	0.286	*0.014*	*0.002*	*0.102*	*0.015*	*0.004*	*0.257*	*0.138*	*0.364*	*0.028*	*0.035*	*0.318*	*0.073*	*0.152*
Self-reported pain measures														
Neck pain intensity_M	0.112	-0.109	-0.081	-0.186	-0.392	-0.397	-0.104	-0.249	-0.125	-0.285	-0.384	-0.317	-0.222	-0.102
	0.593	*0.605*	*0.701*	*0.374*	*0.053*	*0.049*	*0.619*	*0.230*	*0.550*	*0.168*	*0.058*	*0.122*	*0.285*	*0.628*
Maladaptive pain cognitions														
PCS	-0.024	-0.400	-0.397	-0.232	**-0.535**	-0.355	-0.215	**-0.515**	-0.153	-0.412	-0.482	-0.322	-0.221	-0.210
	0.91	*0.047*	*0.049*	*0.265*	*0.006*	*0.082*	*0.303*	*0.008*	*0.466*	*0.041*	*0.015*	*0.116*	*0.287*	*0.313*
PVAQ	-0.277	-0.501	**-0.657**	-0.243	-0.441	-0.249	-0.088	-0.420	-0.152	-0.384	-0.325	-0.265	0.101	-0.199
	0.18	*0.011*	*<0.001*	*0.241*	*0.027*	*0.230*	*0.676*	*0.037*	*0.468*	*0.058*	*0.113*	*0.200*	*0.631*	*0.340*
Self-reported symptoms of central sensitization														
CSI	-0.246	-0.436	-0.317	-0.283	-0.406	-0.137	-0.154	-0.212	-0.365	-0.440	-0.421	-0.123	-0.444	-0.105
	0.247	*0.033*	*0.131*	*0.181*	*0.049*	*0.524*	*0.471*	*0.320*	*0.080*	*0.031*	*0.040*	*0.568*	*0.030*	*0.625*
Local hyperalgesia														
PPT trapezius	0.232	0.271	0.202	-0.012	0.437	-0.066	0.002	0.345	0.409	0.336	0.107	0.361	0.035	0.091
	0.265	*0.190*	*0.334*	*0.953*	*0.029*	*0.753*	*0.994*	*0.091*	*0.042*	*0.100*	*0.610*	*0.076*	*0.870*	*0.666*
Distant hyperalgesia														
PPT quadriceps	0.063	0.022	0.187	0.098	0.427	-0.053	-0.114	0.004	0.252	0.139	0.113	0.154	0.168	0.094
	0.763	*0.919*	*0.371*	*0.642*	*0.033*	*0.801*	*0.588*	*0.985*	*0.225*	*0.507*	*0.592*	*0.463*	*0.421*	*0.655*
CPM efficacy														
CPM quadriceps	-0.093	0.086	-0.041	-0.003	-0.026	-0.205	-0.063	0.232	0.409	0.349	0.084	0.253	-0.097	0.177
	0.689	*0.712*	*0.860*	*0.989*	*0.910*	*0.372*	*0.786*	*0.312*	*0.066*	*0.120*	*0.717*	*0.268*	*0.676*	*0.442*

Significant correlations are presented in bold. Correlations significant at the 0.05 level (2-tailed) were not deemed significant in order to correct for multiple comparisons. Correlations significant at the 0.01 level (2-tailed) were deemed significant. P-values are presented below the correlation coefficient.

Abbreviations: ACC= anterior cingulate cortex, PCC= posterior cingulate cortex, OBF= orbitofrontal, CWAD= chronic whiplash associated disorders, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, M= MRI test moment, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire, CSI= central sensitization inventory, PPT= pressure pain thresholds, CPM= conditioned pain modulation, RH= right hemisphere.

Moderate correlations were demonstrated between higher levels of pain catastrophizing, and decreased GMV of the left precuneus (r_s= -0.522; p=0.007), the left pars orbitalis (r_s= -0.560; p=0.004), the right medial orbitofrontal cortex (r_s= -0.535; p=0.006), and the right insula (r_s= -0.515; p=0.008). Furthermore, moderate correlations were found between higher levels of pain hypervigilance, and decreased GMV of the left rostral middle frontal cortex (r_s= -0.576; p=0.003), the left thalamus (r_s= -0.572; p=0.003), and the right posterior cingulate cortex (r_s= -0.657; p<0.001). A moderate relationship was observed between more self-perceived CS symptoms, and decreased GMV of the left amygdala (r_s= -0.636; p=0.001).

Moreover, a moderate relationship was found between lower PPTs at the trapezius muscle, and decreased GMV of the left postcentral cortex (r_s= 0.551; p=0.004). Finally, no significant correlations were detected between regional GMV and efficacy of CPM (p>0.01).

Discussion

The results of the present innovative study provided evidence for decreased GMV in cortical regions known to be associated with processing cognition and pain in patients with CWAD compared to CINP patients and healthy persons. In contrast, regional GMV alterations were not observed in CINP patients compared to healthy persons. Furthermore, this study revealed for the first time that increased cognitive deficits, maladapted pain cognitions, CS symptoms, and local hyperalgesia were moderately correlated with decreased regional GMV in CWAD patients. In CINP patients, regional GMV was only correlated with cognitive deficits.

Group differences in regional GMV

The observed cortical GMV decrease in patients with CWAD compared to CINP patients and healthy controls was in line with our hypothesis and could be explained because CWAD patients have a traumatic origin of neck pain and are characterized by CS in contrast to CINP patients, who have a non-traumatic origin of neck pain, and do not show CS at a group level. In the present study, decreased GMV was demonstrated in the left posterior cingulate cortex, and the right superior parietal cortex in patients with CWAD compared to CINP. This is the first study investigating and revealing these regional GMV differences between women with CINP and CWAD. Compared to healthy women, decreased GMV could also be revealed in the left posterior cingulate cortex, right lateral orbitofrontal cortex, and left supramarginal cortex in women with CWAD. These findings are in line with accumulating evidence of decreased regional GMV in other chronic pain populations characterized by CS such as fibromyalgia and chronic low back pain [19-21, 90, 91]. In particular, decreased GMV has previously been observed in the posterior cingulate cortex, lateral orbitofrontal cortex, and supramarginal cortex in the latter chronic pain populations compared to healthy persons [19, 20, 84, 92-95].

One previous study in patients who developed chronic headache after a whiplash injury, also observed regional GMV decrease however in the anterior cingulate cortex and the dorsolateral prefrontal cortex in the patient group compared to healthy controls [96]. To our knowledge, only one previous study has examined GMV alterations in patients with non-traumatic chronic neck pain, more specifically in patients with chronic myofascial pain resulting from active trigger points in the trapezius muscle. The authors found decreased GMV in the left parahippocampal cortex, and the right fusiform cortex in the patient group compared to healthy persons [97]. Despite these promising results, the authors did not correct for multiple comparisons.

Nevertheless, contrary to previous evidence regarding GMV alterations in regions such as the insula, anterior cingulate cortex, and amygdala in other chronic pain patients, we could not find GMV alterations in all other ROIs. Although, this can be due to methodological factors (e.g. MRI acquisition parameters, MRI processing, poor control in previous studies for age, pain duration, and comorbidities [98]), unique pathology-specific GM morphology alterations in different chronic pain types [86, 92] may also account for these differences.

On the basis of the results of a quantitative meta-analysis investigating GMV alterations in patients with chronic pain, the observed estimated mean differences in regional GMV (mm^3) of the present study in patients with CWAD compared to healthy controls are rather comparable with the results of GMV changes in other chronic pain patients [84]. However, caution is warranted when comparing results of studies that applied different MRI acquisition, analyzing, and processing techniques (e.g. FreeSurfer versus voxel-based morphometry).

Group differences in measures of cognition, pain, and CS

Furthermore, patients with CWAD displayed higher neck pain intensity, more severe pain-related disability, more pronounced cognitive deficits, and more signs of CS compared to patients with non-traumatic CINP. One previous study comparing CINP and CWAD patients, also observed significant features of CS in CWAD patients and not in patients with CINP [99]. Moreover, higher levels of pain catastrophizing and hypervigilance were only present in the CWAD group compared to healthy persons. Accordingly, based on the present study results, it is plausible that more severe cognitive deficits and disturbed pain processing in CWAD patients are associated with a larger extent of maladapted GM morphology reorganization compared to patients with CINP.

Relationships between regional GMV and measures of cognition, pain, and CS

The results of our correlation analyses have demonstrated relationships between decreased regional GMV and debilitating symptoms in CWAD patients. In particular, we revealed that decreased GMV in cognitive and pain processing regions (left pars orbitalis,

left amygdala, left rostral middle frontal cortex, lateral orbitofrontal cortex bilateral, insula bilateral, left precuneus, left thalamus, left postcentral cortex, right medial orbitofrontal cortex, right rostral anterior cingulate cortex, right posterior cingulate cortex) coincided with increased cognitive deficits, maladapted pain cognitions, CS symptoms, and local hyperalgesia in CWAD. Noteworthy, in CINP patients decreased GMV (left rostral anterior cingulate cortex, left medial orbitofrontal cortex, right thalamus) was only associated with increased cognitive deficits but not with pain cognitions, CS symptoms, and local hyperalgesia. The present study could not detect relationships between CPM efficacy and regional GMV in both chronic neck pain groups, in contrast to a previous morphological MRI study in patients with irritable bowel syndrome [100]. This study revealed a relationship between endogenous pain inhibition and cortical thickness in the lateral orbitofrontal cortex. This inconsistent result could be, however, explained by a different CPM paradigm and a different macrostructural metric (cortical thickness versus GMV) [100].

Remarkably, only GMV of the right lateral orbitofrontal cortex was sensitive in detecting significant group differences and was also correlated with measures of cognition and pain. Specifically, decreased GMV in the right lateral orbitofrontal cortex in CWAD patients correlated with worse executive control. Functional neuroimaging combined with neuropsychological data have provided evidence which indicates an important role of the orbitofrontal cortex in decision-making [101] and executive control of information processing by inhibiting neural activity associated with painful sensations [102].

Furthermore, the present study showed associations between increased self-reported cognitive deficits and worse objective cognitive performance (working memory capacity, task-switching capacity, and executive control), and decreased regional GMV in CINP and CWAD patients. Similarly, Luerding et al [24] demonstrated associations between reduced working memory performance and decreased GMV in the left dorsolateral prefrontal cortex in fibromyalgia patients.

Higher levels of pain catastrophizing and pain hypervigilance were correlated with decreased GMV in the precuneus, inferior frontal gyrus (pars orbitalis), medial orbitofrontal cortex, insula, thalamus, posterior cingulate cortex, and rostral middle frontal cortex in patients with CWAD. Our results are consistent with previous studies exploring associations between pain catastrophizing and GM morphology. For example, Hubbard et al [103] observed associations between higher pain catastrophizing and lower GMV in pain processing regions in migraine patients.

Furthermore, increased local hyperalgesia as revealed by lower PPTs at the trapezius muscle in CWAD was correlated with decreased GMV in the left postcentral cortex, which is a region involved in pain perception and processing nociceptive stimuli [104]. Recently, Niddam et al [97] demonstrated an association between decreased PPTs at the trapezius muscle (local hyperalgesia) and decreased GMV in the right middle frontal cortex in patients with chronic myofacial pain.

Lastly, our study found that increased self-reported symptoms of CS were correlated with decreased GMV in the left amygdala in CWAD. Interestingly, the amygdala is a key region involved in pain processing and cognitive factors of pain anticipation [105], and has a crucial role in negative emotions and pain-related memories [106].

Limitations and Strengths

With regard to interpretation of the present study results, the following limitations must be taken into account. First, the cross-sectional nature of this study implies that no conclusions about the causality of the observed relationships can be drawn. Second, the generalizability of the study results might be reduced because only women were included and only CWAD patients classified as WAD II A, B or C, but, this results in less heterogeneity in the included study sample which is also a strength.

However, the present study also had several strengths. First, this study is the first to address the relationships between GMV alterations on one hand, and self-reported and experimental features of cognition, pain, and CS on the other hand in CINP and CWAD. Second, all groups were comparable in age, body mass index, education level, smoking status, menstrual phase, medication use, neck pain duration, and frequency of neck pain (for the patient groups). In addition, the researchers anticipated sources of bias such as use of medications, caffeine, alcohol, and nicotine on the assessment days. A final strength is the use of FreeSurfer, which has some advantages over voxel-based morphometry.

Clinical message

Our results indicated that chronic pain in CWAD patients should be interpreted, at least in part, as a result of neural reorganization of the CNS associated with alterations in GMV of regions involved in various aspects of pain and cognitive processing.

Importantly, increased cognitive deficits, maladapted pain cognitions, and CS symptoms were found to be associated with decreased GMV in regions implicated in processing cognition and pain in CWAD patients. Therefore, it can be recommended that therapy approaches for CWAD should address the brain and take into account neuroplasticity of the CNS. Cognitive behavioral therapy can be advocated and has been demonstrated to reverse regional GMV decreases associated with reduced pain catastrophizing and decreased cognitive deficits in other chronic pain patients characterized by CS [107, 108].

In CINP patients, only cognitive deficits were related to decreased regional GMV, and no GMV alterations or CS could be revealed. Accordingly, fewer indications are currently available for a role of brain alterations and CNS reorganization in the pathophysiology of CINP at a group level. Nevertheless, at the individual patient level it is still possible that CNS mechanisms play a role, and subsequently the therapeutic approach should be personalized for each specific patient regardless of diagnosis.

Encouragingly, multiple studies have shown in other chronic pain conditions that decrease in GM morphology including GMV is at least partially reversible when underlying pain is adequately treated [96, 109, 110]. These studies are clinically relevant as they suggest that at least some of the morphological GM changes must be a direct consequence of the presence of pain, and related sequel, and possibly the underlying mechanism is based on synaptic plasticity [93].

To summarize, the current study results pave the way for the development of novel and more effective treatment approaches for patients with chronic neck pain.

Recommendations for further research

The exact underlying mechanisms responsible for decreased regional GMV in CWAD patients remain unclear. The potential underlying mechanisms for GMV changes include changes in synaptic density and dendritic spine structure, among others [111]. It is possible that the observed GMV decrease reflects tissue shrinkage, which can be caused by affected neural tissue or extracellular and microvascular volume without substantially impacting neuronal properties [112]. Further research should investigate the underlying neurobiological mechanisms of the observed GMV alterations. In addition, future research should investigate possible alterations in white matter microstructure in patients with CWAD compared to CINP.

The regional GMV decrease can also be interpreted in the light of maladapted neuroplasticity [113]. This is relevant when considering the dynamic features of GMV alterations associated with persistent pain. Neural reorganization can range from synaptic plasticity to changes in neural circuitry (e.g. long-term potentiation, synaptic sprouting, neurogenesis [114], and glial reorganization).

Whether these GMV changes are the consequence of pain or whether pre-existent alterations of these regions make patients more susceptible to the development of CWAD remains to be elucidated. Longitudinal research is warranted and research should unravel if therapy can re-shape the brain and diminish the associated burden in CWAD. Noteworthy, the current study has investigated only one piece of the puzzle regarding possible brain alterations in patients with CINP and CWAD. Accordingly, future brain imaging research has to further disentangle possible structural and functional brain changes in patients with chronic neck pain.

Conclusion

The present innovative study provided evidence for decreased GMV in cortical regions associated with pain and cognitive processing in women with CWAD compared to women with CINP and healthy women. Additionally, in women with CWAD, decreased GMV in cognitive and pain processing regions was associated with increased cognitive deficits, maladapted pain cognitions, self-perceived CS symptoms, and local hyperalgesia. In women with CINP, decreased GMV was only associated with increased cognitive deficits but compared with healthy controls no GMV alterations could be revealed. These findings indicate a possible negative mediating role of the trauma in patients with CWAD. The underlying neurobiological mechanisms of these GMV alterations remain to be elucidated and no conclusions about the causality of the observed relationships can be drawn. Accordingly, longitudinal research is warranted to unravel whether these GMV alterations occur as a result of chronic pain or vice versa. Based on the present study results, it can be recommended that therapy approaches for CWAD should take into account the role of CNS neuroplasticity.

Acknowledgements

The authors gratefully acknowledge the participants of this study, without whom this study could have never been completed successfully. For its financial support, the authors' gratitude goes out to Special Research Fund of Ghent University (BOF-Ghent) and the Agency for Innovation by Science and Technology (IWT), who made this experimental research possible. Finally, we acknowledge Stephanie Bogaert for her radiological assistance with the T2*-weighted MRI analyses, and we acknowledge the neuroradiological expertise of Dr. Karel Deblaere and Dr. Eva Genbrugge to visually inspect all the T2*-weighted brain images.

Supplementary Table A Differences in GMV of ROIs involved in processing of cognition and pain in CWAD patients, CINP patients and healthy women.

Estimated means					Tests of between-Subjects Effects							
	Mean*	Std. Error	95% Confidence Interval		F-value	P-value	Pairwise comparisons Adjustment for multiple comparisons: Bonferroni.					
			Lower Bound	Upper Bound				Estimated Mean Difference	Std. Error	*P*-value	95% Confidence Interval for Difference	
											Lower Bound	Upper Bound
Posterior cingulate cortex volume (left hemisphere) (mm³)												
HCON	3267.763	82.534	3103.418	3432.108	4.213	**0.018**	**HCON-CINP**	6.072	111.036	1.000	-265.678	277.823
CINP	3261.691	73.356	3115.621	3407.760			**HCON-CWAD**	289.223	116.929	**0.047**	3.051	575.394
CWAD	2978.540	81.757	2815.742	3141.339			**CINP-CWAD**	283.150	109.525	**0.035**	15.099	551.202
Lateral orbitofrontal cortex volume (right hemisphere) (mm³)												
HCON	6895.475	125.968	6644.641	7146.310	4.103	**0.020**	**HCON-CINP**	129.357	169.471	1.000	-285.407	544.121
CINP	6766.118	111.960	6543.177	6989.060			**HCON-CWAD**	488.418	178.464	**0.023**	51.643	925.192
CWAD	6407.058	124.783	6158.583	6655.532			**CINP-CWAD**	359.061	167.164	0.105	-50.057	768.179
Supramarginal cortex volume (left hemisphere) (mm³)												
HCON	11399.953	264.636	10872.995	11926.911	4.504	**0.014**	**HCON-CINP**	705.567	356.027	0.153	-165.776	1576.910
CINP	10694.386	235.208	10226.027	11162.745			**HCON-CWAD**	1112.305	374.921	**0.012**	194.722	2029.888
CWAD	10287.648	262.146	9765.649	10809.648			**CINP-CWAD**	406.738	351.181	0.751	-452.744	1266.220
Superior parietal cortex volume (right hemisphere) (mm³)												
HCON	12753.563	269.306	12217.305	13289.820	4.839	**0.010**	**HCON-CINP**	-492.403	362.31	0.534	-1379.124	394.317
CINP	13245.966	239.359	12769.342	13722.590			**HCON-CWAD**	619.364	381.537	0.326	-314.413	1553.140
CWAD	12134.199	266.772	11602.988	12665.411			**CINP-CWAD**	1111.767	357.379	**0.008**	237.117	1986.417
Total Intracranial volume (mm³)												
HCON	1455072.696	35504.319	1384374.565	1525770.826	2.284	0.109	**HCON-CINP**	NA				
CINP	1369525.631	31556.148	1306689.312	1432361.949			**HCON-CWAD**					
CWAD	1357070.762	35170.249	1287037.849	1427103.675			**CINP-CWAD**					
Total grey matter volume (mm³)												
HCON	626666.100	8789.236	609164.496	644167.704	1.655	0.198	**HCON-CINP**	NA				
CINP	620007.734	7811.850	604452.353	635563.115			**HCON-CWAD**					
CWAD	604746.527	8706.535	587409.601	622083.454			**CINP-CWAD**					
Subcortical volume (mm³)												
HCON	56059.367	670.566	54724.098	57394.635	0.680	0.510	**HCON-CINP**	NA				
CINP	56311.981	595.998	55125.198	57498.764			**HCON-CWAD**					
CWAD	55297.537	664.257	53974.832	56620.241			**CINP-CWAD**					

*Covariates appearing in the model are evaluated at the following values: age = 33.630
HCON n= 25; CINP n= 31; CWAD n=25
Abbreviations: HCON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, ROIs= regions of interest, NA= not applicable

Supplementary Table B Non-significant differences in GMV of ROIs involved in processing of cognition and pain in CWAD patients, CINP patients and healthy women.

Estimated means					Tests of between-Subjects Effects
	Mean*	Std. Error	95% Confidence Interval		*F*-value (*P*-value)
			Lower Bound	Upper Bound	
Caudal anterior cingulate volume (left hemisphere) (mm³)					
HCON	2022.697	84.968	1853.504	2191.890	1.146 (0.323)
CINP	1849.925	75.519	1699.546	2000.303	
CWAD	1933.957	84.169	1766.356	2101.558	
Caudal anterior cingulate volume (right hemisphere) (mm³)					
HCON	2238.221	88.037	2062.916	2413.526	0.662 (0.519)
CINP	2103.819	78.247	1948.008	2259.629	
CWAD	2182.124	87.209	2008.469	2355.779	
Rostral anterior cingulate volume (left hemisphere) (mm³)					
HCON	2815.528	95.081	2626.197	3004.859	1.857 (0.163)
CINP	2595.038	84.508	2426.761	2763.314	
CWAD	2592.465	94.187	2404.916	2780.015	
Rostral anterior cingulate volume (right hemisphere) (mm³)					
HCON	2223.509	78.969	2066.262	2380.756	1.058 (0.352)
CINP	2148.890	70.187	2009.129	2288.650	
CWAD	2061.148	78.226	1905.381	2216.915	
Posterior cingulate volume (right hemisphere) (mm³)					
HCON	3239.293	87.813	3064.435	3414.151	1.755 (0.180)
CINP	3080.014	78.048	2924.600	3235.427	
CWAD	3012.570	86.987	2839.357	3185.782	
Rostral middle frontal volume (left hemisphere) (mm³)					
HCON	15797.621	340.979	15118.645	16476.597	1.027 (0.363)
CINP	15447.520	303.061	14844.048	16050.992	
CWAD	15105.454	337.771	14432.867	15778.042	
Rostral middle frontal volume (right hemisphere) (mm³)					
HCON	15021.037	343.129	14337.780	15704.294	0.347 (0.708)
CINP	14776.898	304.972	14169.621	15384.174	
CWAD	14618.770	339.900	13941.942	15295.598	
Medial orbitofrontal volume (left hemispere) (mm³)					
HCON	4624.112	108.906	4407.251	4840.973	2.305 (0.107)
CINP	4540.005	96.796	4347.260	4732.750	
CWAD	4307.561	107.882	4092.741	4522.381	
Medial orbitofrontal volume (right hemispere) (mm³)					
HCON	4786.377	97.557	4592.116	4980.637	1.228 (0.298)
CINP	4583.517	86.708	4410.859	4756.175	
CWAD	4701.462	96.639	4509.030	4893.895	

Supplementary Table B Continued.

Estimated means					Tests of between-Subjects Effects
	Mean*	Std. Error	95% Confidence Interval		*F*-value (*P*-value)
			Lower Bound	Upper Bound	
Lateral orbitofrontal volume (left hemisphere) (mm³)					
HCON	7556.569	150.887	7256.115	7857.023	1.161 (0.319)
CINP	7396.945	134.108	7129.902	7663.988	
CWAD	7230.979	149.467	6933.351	7528.606	
Insula volume (left hemisphere) (mm³)					
HCON	6650.029	135.783	6379.651	6920.407	1.807 (0.171)
CINP	6619.807	120.683	6379.495	6860.118	
CWAD	6326.050	134.505	6058.216	6593.884	
Insula volume (right hemisphere) (mm³)					
HCON	6759.804	131.606	6497.743	7021.865	1.419 (0.248)
CINP	6677.191	116.971	6444.272	6910.110	
CWAD	6458.759	130.368	6199.164	6718.355	
Postcentral volume (left hemisphere) (mm³)					
HCON	9350.764	227.937	8896.884	9804.645	0.590 (0.557
CINP	9683.044	202.590	9279.637	10086.452	
CWAD	9517.861	225.792	9068.251	9967.471	
Postcentral volume (right hemisphere) (mm³)					
HCON	9045.141	267.322	8512.835	9577.447	0.028 (0.973)
CINP	8984.258	237.595	8511.146	9457.370	
CWAD	8958.659	264.807	8431.362	9485.957	
Precuneus volume (left hemisphere) (mm³)					
HCON	9579.739	220.170	9141.325	10018.153	1.011 (0.369)
CINP	9593.422	195.686	9203.761	9983.083	
CWAD	9214.218	218.098	8779.929	9648.506	
Precuneus volume (right hemisphere) (mm³)					
HCON	992.580	200.089	9530.152	10327.008	2.240 (0.113)
CINP	10015.495	177.838	9661.373	10369.617	
CWAD	9478.566	198.206	9083.887	9873.245	
Pars Orbitalis volume (left hemisphere) (mm³)					
HCON	2201.797	56.529	2089.233	2314.361	1.609 (0.207)
CINP	2218.293	50.243	2118.247	2318.340	
CWAD	2091.079	55.997	1979.574	2202.584	
Pars Orbitalis volume (right hemisphere) (mm³)					
HCON	2649.025	71.052	2507.542	2790.509	1.406 (0.251)
CINP	2580.656	63.151	2454.906	2706.406	
CWAD	2481.601	70.384	2341.449	2621.753	

Supplementary Table B Continued.

Estimated means					Tests of between-Subjects Effects
	Mean*	Std. Error	95% Confidence Interval		*F*-value (*P*-value)
			Lower Bound	Upper Bound	
Supramarginal cortex volume (right hemisphere) (mm³)					
HCON	10579.946	287.451	10007.558	11152.334	1.700 (0.190)
CINP	10192.171	255.486	9683.434	10700.908	
CWAD	9829.081	284.746	9262.079	10396.084	
Superior parietal volume (left hemisphere) (mm³)					
HCON	13092.699	301.360	12492.614	13692.784	1.212 (0.303)
CINP	13000.016	267.848	12466.663	13533.370	
CWAD	12486.401	298.525	11891.962	13080.839	
Amygdala volume (left hemisphere) (mm³)					
HCON	1502.540	33.352	1436.128	1568.952	2.428 (0.095)
CINP	1601.342	29.643	1542.315	1660.369	
CWAD	1555.476	33.038	1489.689	1621.263	
Amygdala volume (right hemisphere) (mm³)					
HCON	1464.975	29.053	1407.123	1522.827	1.652 (0.198)
CINP	1535.188	25.822	1483.769	1586.607	
CWAD	1514.461	28.780	1457.153	1571.768	
Thalamus volume (left hemisphere) (mm³)					
HCON	7818.711	170.216	7479.768	8157.655	1.043 (0.357)
CINP	7792.152	151.288	7490.900	8093.404	
CWAD	7511.332	168.615	7175.578	7847.087	
Thalamus volume (right hemisphere) (mm³)					
HCON	7046.965	117.792	6812.411	7281.518	1.947 (0.150)
CINP	6999.345	104.693	6790.875	7207.816	
CWAD	6745.843	116.683	6513.497	6978.189	

*Covariates appearing in the model are evaluated at the following values: age = 33.630.
HCON n= 25; CINP n= 31; CWAD n=25
Abbreviations: HCON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, ROIs= regions of interest

References

1. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J* 2006; 15:834-848.
2. Murray CJ, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013; 369:448-457.
3. Verhagen AP, Scholten-Peeters GG, van Wijngaarden S, de Bie RA, Bierma-Zeinstra SM. Conservative treatments for whiplash. *Cochrane Database Syst Rev* 2007:Cd003338.
4. Borghouts JA, Koes BW, Bouter LM. The clinical course and prognostic factors of non-specific neck pain: a systematic review. *Pain* 1998; 77:1-13.
5. Barnsley L, Lord S, Bogduk N. Whiplash injury. *Pain* 1994; 58:283-307.
6. Coppieters I., De Pauw R., Kregel J., Malfliet A., Goubert D., Lenoir D., Cagnie B., M. M. Differences between women with traumatic and idiopathic chronic neck pain and women without neck pain: interrelationships among disability, cognitive deficits, and central sensitization. *Physical Therapy* March 2017.
7. Davis CG. Mechanisms of chronic pain from whiplash injury. *J Forensic Leg Med* 2013; 20:74-85.
8. Elliott JM, Noteboom JT, Flynn TW, Sterling M. Characterization of acute and chronic whiplash-associated disorders. *J Orthop Sports Phys Ther* 2009; 39:312-323.
9. Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. *Clin J Pain* 2005; 21:175-181.
10. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician* 2015; 18:E389-401.
11. Borsbo B, Peolsson M, Gerdle B. Catastrophizing, depression, and pain: correlation with and influence on quality of life and health - a study of chronic whiplash-associated disorders. *J Rehabil Med* 2008; 40:562-569.
12. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013; 17:299-312.
13. Malfliet A, Kregel J, Cagnie B, Kuipers M, Dolphens M, Roussel N, Meeus M, Danneels L, Bramer WM, Nijs J. Lack of evidence for central sensitization in idiopathic, non-traumatic neck pain: a systematic review. *Pain Physician* 2015; 18:223-236.
14. Dimitriadis Z, Kapreli E, Strimpakos N, Oldham J. Do psychological states associate with pain and disability in chronic neck pain patients? *J Back Musculoskelet Rehabil* 2015; 28:797-802.
15. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain* 2011; 152:S49-64.
16. Schmidt-Wilcke T. Neuroimaging of chronic pain. *Best Pract Res Clin Rheumatol* 2015; 29:29-41.
17. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 2015; 87:474-491.
18. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. *Journal of Neuroimmune Pharmacology* 2013; 8:518-534.
19. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Semin Arthritis Rheum* 2014; 44:68-75.
20. Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B. Structural and functional brain abnormalities in chronic low back pain: A systematic review. *Semin Arthritis Rheum* 2015.
21. Younger JW, Shen YF, Goddard G, Mackey SC. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. *Pain* 2010; 149:222-228.
22. Mordasini L, Weisstanner C, Rummel C, Thalmann GN, Verma RK, Wiest R, Kessler TM. Chronic pelvic pain syndrome in men is associated with reduction of relative gray matter volume in the anterior cingulate cortex compared to healthy controls. *J Urol* 2012; 188:2233-2237.
23. Moayedi M, Weissman-Fogel I, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. *Neuroimage* 2011; 55:277-286.
24. Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain* 2008; 131:3222-3231.

25. McAllister SJ, Toussaint LL, Williams DA, Hoskin TL, Whipple MO, Vincent A. Perceived dyscognition reported by patients with fibromyalgia. *Clin Exp Rheumatol* 2016; 34:S48-54.
26. Tamburin S, Maier A, Schiff S, Lauriola MF, Di Rosa E, Zanette G, Mapelli D. Cognition and emotional decision-making in chronic low back pain: an ERPs study during Iowa gambling task. *Front Psychol* 2014; 5:1350.
27. Schierz O, Nixdorf DR, Singer S, Reissmann DR. Self-reported ability to concentrate in patients with painful temporomandibular disorders compared to the general population. *Community Dent Oral Epidemiol* 2012; 40:507-515.
28. Zusman M. Forebrain-mediated sensitization of central pain pathways: 'non-specific' pain and a new image for MT. *Man Ther* 2002; 7:80-88.
29. Blankstein U, Chen J, Diamant NE, Davis KD. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology* 2010; 138:1783-1789.
30. Killgore WD, Singh P, Kipman M, Pisner D, Fridman A, Weber M. Gray matter volume and executive functioning correlate with time since injury following mild traumatic brain injury. *Neurosci Lett* 2016; 612:238-244.
31. Moshourab RA, Schafer M, Al-Chaer ED. Frontiers in Neuroengineering Chronic Pain in Neurotrauma: Implications on Spinal Cord and Traumatic Brain Injury, in Kobeissy FH (ed). Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. CRC Press/Taylor & Francis (c) 2015 by Taylor & Francis Group, LLC., Boca Raton (FL), 2015.
32. Nampiaparampil DE. Prevalence of chronic pain after traumatic brain injury: a systematic review. *Jama* 2008; 300:711-719.
33. Bay E, Kalpakjian C, Giordani B. Determinants of subjective memory complaints in community-dwelling adults with mild-to-moderate traumatic brain injury. *Brain Inj* 2012; 26:941-949.
34. Dean PJ, Sterr A. Long-term effects of mild traumatic brain injury on cognitive performance. *Front Hum Neurosci* 2013; 7:30.
35. Jamora CW, Young A, Ruff RM. Comparison of subjective cognitive complaints with neuropsychological tests in individuals with mild vs more severe traumatic brain injuries. *Brain Inj* 2012; 26:36-47.
36. Erez AB, Rothschild E, Katz N, Tuchner M, Hartman-Maeir A. Executive functioning, awareness, and participation in daily life after mild traumatic brain injury: a preliminary study. *Am J Occup Ther* 2009; 63:634-640.
37. Ruigrok AN, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev* 2014; 39:34-50.
38. Maleki N, Linnman C, Brawn J, Burstein R, Becerra L, Borsook D. Her versus his migraine: multiple sex differences in brain function and structure. *Brain* 2012; 135:2546-2559.
39. Good CD, Johnsrude I, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage* 2001; 14:685-700.
40. Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. *J Pain Res* 2015; 8:311-320.
41. Popescu A, LeResche L, Truelove EL, Drangsholt MT. Gender differences in pain modulation by diffuse noxious inhibitory controls: a systematic review. *Pain* 2010; 150:309-318.
42. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 2006; 10:287-333.
43. Vernon H. The Neck Disability Index: state-of-the-art, 1991-2008. *J Manipulative Physiol Ther* 2008; 31:491-502.
44. Sterling M. A proposed new classification system for whiplash associated disorders--implications for assessment and management. *Man Ther* 2004; 9:60-70.
45. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine (Phila Pa 1976)* 1995; 20:1s-73s.
46. Rao V, Lyketsos C. Neuropsychiatric sequelae of traumatic brain injury. *Psychosomatics* 2000; 41:95-103.
47. Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. *J Manipulative Physiol Ther* 1991; 14:409-415.
48. Jorritsma W, de Vries GE, Geertzen JH, Dijkstra PU, Reneman MF. Neck Pain and Disability Scale and the Neck Disability Index: reproducibility of the Dutch Language Versions. *Eur Spine J* 2010; 19:1695-1701.

49. Jorritsma W, de Vries GE, Dijkstra PU, Geertzen JH, Reneman MF. Neck Pain and Disability Scale and Neck Disability Index: validity of Dutch language versions. *Eur Spine J* 2012; 21:93-100.
50. Ailliet L, Rubinstein SM, de Vet HC, van Tulder MW, Terwee CB. Reliability, responsiveness and interpretability of the neck disability index-Dutch version in primary care. *Eur Spine J* 2015; 24:88-93.
51. Jorritsma W, de Vries GE, Dijkstra PU, Geertzen JHB, Reneman MF. Neck Pain and Disability Scale and Neck Disability Index: validity of Dutch language versions. *European Spine Journal* 2012; 21:93-100.
52. Takasaki H, Chien CW, Johnston V, Treleaven J, Jull G. Validity and reliability of the perceived deficit questionnaire to assess cognitive symptoms in people with chronic whiplash-associated disorders. *Arch Phys Med Rehabil* 2012; 93:1774-1781.
53. Sanchez-Cubillo I, Perianez JA, Adrover-Roig D, Rodriguez-Sanchez JM, Rios-Lago M, Tirapu J, Barcelo F. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *J Int Neuropsychol Soc* 2009; 15:438-450.
54. Osman A, Barrios FX, Kopper BA, Hauptmann W, Jones J, O'Neill E. Factor structure, reliability, and validity of the Pain Catastrophizing Scale. *J Behav Med* 1997; 20:589-605.
55. Lame IE, Peters ML, Kessels AG, Van Kleef M, Patijn J. Test--retest stability of the Pain Catastrophizing Scale and the Tampa Scale for Kinesiophobia in chronic pain over a longer period of time. *J Health Psychol* 2008; 13:820-826.
56. Van Damme S, Crombez, G., Vlaeyen, J. W. S., Goubert, L., Van den Broeck, A., Van Houdenhove, B. De Pain Catastrophizing Scale: Psychometrische karakteristieken en normen. *Gedragstherapie* 2000; 33:209-220.
57. Van Damme S, Crombez G, Bijttebier P, Goubert L, Van Houdenhove B. A confirmatory factor analysis of the Pain Catastrophizing Scale: invariant factor structure across clinical and non-clinical populations. *Pain* 2002; 96:319-324.
58. Roelofs J, Peters ML, Muris P, Vlaeyen JW. Dutch version of the Pain Vigilance and Awareness Questionnaire: validity and reliability in a pain-free population. *Behav Res Ther* 2002; 40:1081-1090.
59. Roelofs J, Peters ML, McCracken L, Vlaeyen JW. The pain vigilance and awareness questionnaire (PVAQ): further psychometric evaluation in fibromyalgia and other chronic pain syndromes. *Pain* 2003; 101:299-306.
60. Kregel J, Vuijk PJ, Descheemaeker F, Keizer D, van der Noord R, Nijs J, Cagnie B, Meeus M, van Wilgen P. The Dutch Central Sensitization Inventory (CSI): Factor Analysis, Discriminative Power and Test-Retest Reliability. *Clin J Pain* 2015.
61. Mayer TG, Neblett R, Cohen H, Howard KJ, Choi YH, Williams MJ, Perez Y, Gatchel RJ. The development and psychometric validation of the central sensitization inventory. *Pain Pract* 2012; 12:276-285.
62. Neblett R, Cohen H, Choi Y, Hartzell MM, Williams M, Mayer TG, Gatchel RJ. The Central Sensitization Inventory (CSI): establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. *J Pain* 2013; 14:438-445.
63. Whiteside A, Hansen S, Chaudhuri A. Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 2004; 109:497-499.
64. Meeus M, Roussel NA, Truijen S, Nijs J. Reduced pressure pain thresholds in response to exercise in chronic fatigue syndrome but not in chronic low back pain: an experimental study. *J Rehabil Med* 2010; 42:884-890.
65. Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. *Scand J Rehabil Med* 1999; 31:89-93.
66. Cathcart S, Pritchard D. Reliability of pain threshold measurement in young adults. *J Headache Pain* 2006; 7:21-26.
67. Ylinen J, Nykanen M, Kautiainen H, Hakkinen A. Evaluation of repeatability of pressure algometry on the neck muscles for clinical use. *Man Ther* 2007; 12:192-197.
68. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol* 2010; 23:611-615.
69. Rehberg B, Baars JH, Kotsch J, Koppe P, von Dincklage F. Comparison of trigeminal and spinal modulation of pain and nociception. *Int J Neurosci* 2012; 122:298-304.
70. Ng TS, Pedler A, Vicenzino B, Sterling M. Less efficacious conditioned pain modulation and sensory hypersensitivity in chronic whiplash-associated disorders in Singapore. *Clin J Pain* 2014; 30:436-442.
71. Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. *Brain Res* 2008; 1230:73-79.

72. Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. *Pain Res Manag* 2012; 17:98-102.
73. Fischl B. FreeSurfer. *Neuroimage* 2012; 62:774-781.
74. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; 33:341-355.
75. Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 2004; 22:1060-1075.
76. Fischl B, Salat DH, van der Kouwe AJ, Makris N, Segonne F, Quinn BT, Dale AM. Sequence-independent segmentation of magnetic resonance images. *Neuroimage* 2004; 23 Suppl 1:S69-84.
77. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998; 17:87-97.
78. Fischl B, Liu A, Dale AM. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging* 2001; 20:70-80.
79. Segonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging* 2007; 26:518-529.
80. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 1999; 9:179-194.
81. Dale AM, Sereno MI. Improved Localizadon of Cortical Activity by Combining EEG and MEG with MRI Cortical Surface Reconstruction: A Linear Approach. *J Cogn Neurosci* 1993; 5:162-176.
82. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 2000; 97:11050-11055.
83. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006; 31:968-980.
84. Smallwood RF, Laird AR, Ramage AE, Parkinson AL, Lewis J, Clauw DJ, Williams DA, Schmidt-Wilcke T, Farrell MJ, Eickhoff SB, Robin DA. Structural Brain Anomalies and Chronic Pain: A Quantitative Meta-Analysis of Gray Matter Volume. *Journal of Pain* 2013; 14:663-675.
85. Coppieters I, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. *J Pain* 2016.
86. Baliki MN, Schnitzer TJ, Bauer WR, Apkarian AV. Brain morphological signatures for chronic pain. *PLoS One* 2011; 6:e26010.
87. Lee DH, Lee KJ, Cho KI, Noh EC, Jang JH, Kim YC, Kang DH. Brain alterations and neurocognitive dysfunction in patients with complex regional pain syndrome. *J Pain* 2015; 16:580-586.
88. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J* 2012; 24:69-71.
89. Perelle IB, Ehrman L. An international study of human handedness: the data. *Behav Genet* 1994; 24:217-227.
90. As-Sanie S, Harris RE, Napadow V, Kim J, Neshewat G, Kairys A, Williams D, Clauw DJ, Schmidt-Wilcke T. Changes in regional gray matter volume in women with chronic pelvic pain: a voxel-based morphometry study. *Pain* 2012; 153:1006-1014.
91. Walitt B, Ceko M, Gracely JL, Gracely RH. Neuroimaging of Central Sensitivity Syndromes: Key Insights from the Scientific Literature. *Curr Rheumatol Rev* 2016; 12:55-87.
92. Cauda F, Palermo S, Costa T, Torta R, Duca S, Vercelli U, Geminiani G, Torta DM. Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. *Neuroimage Clin* 2014; 4:676-686.
93. May A. Chronic pain may change the structure of the brain. *Pain* 2008; 137:7-15.
94. Barad MJ, Ueno T, Younger J, Chatterjee N, Mackey S. Complex regional pain syndrome is associated with structural abnormalities in pain-related regions of the human brain. *J Pain* 2014; 15:197-203.
95. Diaz-Piedra C, Guzman MA, Buela-Casal G, Catena A. The impact of fibromyalgia symptoms on brain morphometry. *Brain Imaging Behav* 2015.
96. Obermann M, Nebel K, Schumann C, Holle D, Gizewski ER, Maschke M, Goadsby PJ, Diener HC, Katsarava Z. Gray matter changes related to chronic posttraumatic headache. *Neurology* 2009; 73:978-983.

97. Niddam DM, Lee SH, Su YT, Chan RC. Brain structural changes in patients with chronic myofascial pain. *Eur J Pain* 2016.
98. Schmidt-Wicke T MA. Anatomical reorganization of the brain with chronic pain, in Apkarian AV (ed). The brain adapting with pain Contribution of neuroimaging technology to pain mechanisms. wolters kluwer, 2015, pp 250.
99. Chien A, Sterling M. Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. *Man Ther* 2010; 15:48-53.
100. Piche M, Chen JI, Roy M, Poitras P, Bouin M, Rainville P. Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls. *J Pain* 2013; 14:1217-1226.
101. Wallis JD. Orbitofrontal cortex and its contribution to decision-making. *Annu Rev Neurosci* 2007; 30:31-56.
102. AP S. The role of the prefrontal cortex in dynamic filtering. . *Psychobiology* 2000; 28:207–218.
103. Hubbard CS, Khan SA, Keaser ML, Mathur VA, Goyal M, Seminowicz DA. Altered Brain Structure and Function Correlate with Disease Severity and Pain Catastrophizing in Migraine Patients. *eNeuro* 2014; 1:e20.14.
104. Ogino Y, Nemoto H, Goto F. Somatotopy in human primary somatosensory cortex in pain system. *Anesthesiology* 2005; 103:821-827.
105. Tracey I. Nociceptive processing in the human brain. *Curr Opin Neurobiol* 2005; 15:478-487.
106. Li Z, Wang J, Chen L, Zhang M, Wan Y. Basolateral amygdala lesion inhibits the development of pain chronicity in neuropathic pain rats. *PLoS One* 2013; 8:e70921.
107. de Lange FP, Koers A, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I. Increase in prefrontal cortical volume following cognitive behavioural therapy in patients with chronic fatigue syndrome. *Brain* 2008; 131:2172-2180.
108. Seminowicz DA, Shpaner M, Keaser ML, Krauthamer GM, Mantegna J, Dumas JA, Newhouse PA, Filippi CG, Keefe FJ, Naylor MR. Cognitive-behavioral therapy increases prefrontal cortex gray matter in patients with chronic pain. *J Pain* 2013; 14:1573-1584.
109. Gwilym SE, Filippini N, Douaud G, Carr AJ, Tracey I. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheum* 2010; 62:2930-2940.
110. Rodriguez-Raecke R, Niemeier A, Ihle K, Ruether W, May A. Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *J Neurosci* 2009; 29:13746-13750.
111. Zatorre RJ, Fields RD, Johansen-Berg H. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat Neurosci* 2012; 15:528-536.
112. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci* 2004; 24:10410-10415.
113. Schmidt-Wicke T MA. Anatomical reorganization of the brain with chronic pain, in Apkarian AV (ed). The brain adapting with pain Contribution of neuroimaging technology to pain mechanisms (ed First). Wolters Kluwer, Philadelphia, 2015, pp 247-248.
114. Bruel-Jungerman E, Davis S, Laroche S. Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 2007; 13:492-505.

Chapter 5

Differences in white matter microstructure and cortical thickness between patients with traumatic and idiopathic chronic neck pain: associations with cognition and pain modulation?

Coppieters I. MSc[1, 2]; De Pauw R. MSc[1]; Caeyenberghs K. PhD[3]; Lenoir D. MSc[1]; DeBlaere K. MD, PhD[4]; Genbrugge E. MD[4]; Meeus M. PhD[1, 2, 5, *]; Cagnie B. PhD[1, *]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium;
[2] Pain in Motion international research group, (www.paininmotion.be);
[3] School of Psychology, Faculty of Health Sciences, Australian Catholic University, Australia;
[4] Department of radiology, Ghent University, Ghent, Belgium;
[5] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium

*Equally contributed last authors

Submitted

Abstract

Background: Alterations in grey matter (GM) and white matter (WM) are hypothesized to be present in patients with chronic whiplash-associated disorders (CWAD). These alterations are possibly associated with the traumatic whiplash injury. Furthermore, these brain alterations are presumed to be associated with more severe cognitive deficits and central sensitization (CS) in CWAD compared to chronic idiopathic neck pain (CINP) patients.

Objectives: To examine alterations in cortical thickness and WM microstructure, and the presence of brain microhemorrhages in a patient group encountering chronic neck pain of traumatic origin (i.e. CWAD) compared to a patient group characterized by non-traumatic chronic neck pain (i.e. CINP), and healthy controls. Furthermore, we aimed to investigate associations between brain structure on one hand and cognitive performance and CS on the other hand.

Methods: T1-weighted, diffusion-weighted and T2*-weighted Magnetic Resonance images of the brain were acquired in 105 women (31 controls, 37 CINP, 37 CWAD) to investigate regional cortical thickness, WM microstructure, and microhemorrhages, respectively. Next, cognitive performance, and CS encompassing distant hyperalgesia and conditioned pain modulation (CPM) efficacy were examined.

Results: We demonstrated worse cognitive performance and decreased CPM efficacy in CWAD compared to CINP patients and controls. Cortical thinning in the left precuneus was revealed in CWAD compared to CINP patients. Also, decreased fractional anisotropy, together with increased values of mean diffusivity and radial diffusivity could be observed in the left cingulum hippocampus and tapetum in CWAD compared to CINP patients, and in the left tapetum in CWAD patients compared to controls. Moreover, the extent of WM microstructural deficits in the left tapetum coincided with decreased CPM efficacy (denoting CS) in the CWAD group.

Conclusion: Alterations in precuneus cortical thickness, and regional WM microstructure were found in women with CWAD, characterized by trauma-induced neck pain. Together, our results provide evidence for associations between decreased endogenous pain inhibition, and the degree of regional WM deficits in CWAD. This yields evidence for underlying WM microstructural correlates of CS, and emphasizes the role of structural brain alterations in women with CWAD compared to CINP. Accordingly, it can be recommended that therapy approaches for CWAD should take into account structural neuroplasticity of the central nervous system.

Keywords: chronic whiplash-associated disorders, chronic idiopathic neck pain, cortical thickness, white matter microstructure, magnetic resonance imaging, central sensitization, cognitive performance

Introduction

Extensive Magnetic Resonance Imaging (MRI) research has uncovered alterations in grey matter (GM) volume, cortical thickness, and white matter (WM) microstructure in regions and tracts involved in processing of pain and cognition in various chronic musculoskeletal pain conditions such as fibromyalgia [1-4] and chronic low back pain [5-7]. In addition, these brain alterations have been reported in mild traumatic brain injury (TBI) patients [8-11]. Also, in mild TBI patients evidence is available for brain microhemorrhages related to the trauma or diffuse axonal injury [12]. Yet, despite this huge body of work, there have been few attempts to investigate these findings in more detail and to examine alterations in both GM and WM, as well as associations with clinical features in patients with chronic musculoskeletal pain.

Remarkably, structural brain MRI research is limited in two prevalent musculoskeletal pain conditions namely chronic whiplash-associated disorders (CWAD), who have trauma-induced persistent neck pain [13], and chronic idiopathic neck pain (CINP), who have persistent neck pain of non-traumatic origin. However, due to the trauma inducing possible shearing forces through acceleration-deceleration of the brain [14], it could be hypothesized that subtle structural brain alterations in GM morphology and WM microstructure are present in CWAD, but not or to a lesser degree in CINP patients. To investigate this hypothesis and to unravel the underlying mechanisms of a wide range of possible structural brain alterations, it seemed valuable to compare patients with CINP and CWAD, a priori different from each other in the origin of pain. Furthermore, this research could explore pathophysiological differences between both chronic neck pain groups which is necessary to improve their treatment outcome.

Intriguingly, many chronic musculoskeletal pain disorders are characterized by central sensitization (CS) as overlapping pathophysiological mechanism, and often experience cognitive problems [15, 16]. In addition, similar to chronic pain patients, mild TBI patients often report cognitive complaints [17] and chronic pain [18]. These findings indicate that chronic pain is associated with structural brain plasticity, and suggest that cognitive deficits and CS are related to this plasticity [19, 20]. Also, it has been shown that GM and WM neuroplasticity is associated with the traumatic factor in mild TBI [8-11].

Compelling evidence has shown CS in patients with CWAD [21]. In contrast, CS is not a characteristic feature in CINP patients [22, 23], and cognitive deficits are observed to a lesser extent compared to CWAD [24]. A recent systematic review concerning brain alterations in WAD and INP, only found 3 studies investigating structural brain alterations exclusively in WAD [25], and one study reported altered GM volume in CWAD. However, alterations in cortical thickness and WM microstructure, and their association with cognitive deficits and CS, have never been investigated in patients with CWAD compared to CINP, which is a prominent research gap.

In order to disentangle subtle structural brain alterations in patients with CWAD compared to non-traumatic CINP, innovative advanced MRI, in particular, Diffusion-Weighted Imaging (DWI) acquisition techniques, and Diffusion Tensor Imaging (DTI) analyses offer opportunities to investigate the brain's WM tissue at a microstructural level [26-31]. Hence, these techniques will probably be more sensitive for detecting subtle structural brain changes in CWAD [32]. In particular, DWI examines WM microstructural organization by quantifying the directionality and degree of diffusion of water within tissues [26-31]. In addition, T2*-weighted MRI could gain insight in the presence or absence of brain microhemorrhages related to the trauma in CWAD. Furthermore, MRI techniques investigating cortical thickness could be valuable to extract additional information of the brain's GM morphology. It is suggested that cortical thickness and GM volume reflect different aspects of the neural architecture [33]. Examining cortical thickness could therefore provide additional insights into the structural neural underpinnings of CWAD and CINP.

The first study objective was to investigate alterations in cortical thickness in brain regions involved in processing of cognition or pain in patients with CWAD compared to CINP and healthy controls. The second objective was to examine deficits in WM microstructure, including alterations in fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) in predefined WM regions/tracts carrying information between regions involved in processing of cognition or pain in patients with CWAD compared to CINP, and healthy controls. The third objective was to examine the presence or absence of microhemorrhages related to trauma in CWAD patients compared to the other study groups. The final objective was to explore in each group separately whether alterations in regional cortical thickness and WM microstructure were associated with cognitive performance and CS.

Based on a-priori cortical thickness and WM anatomical hypotheses, cortical thinning, and deficits in WM microstructure of predefined regions/tracts were hypothesized in CWAD due to the trauma, and because of cognitive deficits [34] and CS [21], compared to CINP patients and healthy controls. Also, previous MRI studies revealed associations between altered cortical thickness and WM microstructure, and measures of cognition and pain in various chronic pain conditions [35, 36]. Therefore, it is hypothesized that alterations in cortical thickness and WM microstructure in CWAD are associated with worse cognitive performance and signs of CS in CWAD compared to CINP patients.

Methods

Participants

Hundred-five female participants - 37 patients with CINP, 37 patients with CWAD, and 31 healthy pain-free controls - were enrolled. A subject sample presenting overlap with the current study sample was used in our recent study with regard to GM volume alterations (unpublished), and concerning clinical differences between women with CINP and CWAD [24]. In order to exclude the confounding factor of gender on brain structure and pain modulation, only women were included, as research has demonstrated differences between men and women regarding brain structure, pain sensitivity and processing in healthy persons and pain patients [37-39]. All participants were Dutch native speakers between 18 to 65 years old. Participants were recruited by calls on social media and through advertisements placed in health magazines and a patient information brochure, on the Ghent University website, and via local radio. Furthermore, informative flyers and posters were distributed in different medical institutes and associations in Flanders, Belgium (various hospitals, medical physician practices, and physical therapist practices). As general inclusion criteria, approximately 90 percent of all participants were right-handed and 10 percent left-handed. This is a representative sample, because approximately 10 percent of the general population is ambidextrous or left-handed [40].

Inclusion criteria for patients with CINP and CWAD were persistent neck pain lasting more than 3 months [41], with a mean pain intensity of more than 3 of 10 on a Numeric Rating Scale (NRS) during the preceding month. All chronic neck pain patients had to report mild/moderate to severe pain-related disability, established by a score of 10 or more of a maximum of 50 on the Neck Disability Index [42]. Additionally, patients with chronic neck pain had to report stability of pain medication intake for at least 4 weeks before study participation.

A specific inclusion criterion for CINP patients was persistent idiopathic neck pain. Patients with CINP were excluded if they ever experienced whiplash trauma, or any other specific cause of neck pain, e.g. cervical hernia with clinical symptoms. Patients with CWAD were included only if they had neck pain resulting from a motor vehicle crash or traumatic event and classifiable as WAD II A, B, or C on the modified Quebec Task Force Scale [43]. Patients with CWAD grades I, III (neurological signs), or IV (fracture or dislocation) on the modified Quebec Task Force Scale were excluded. Additionally, CWAD patients who lost consciousness as a result of the traumatic event, and who had suffered distinct posttraumatic amnesia were excluded.

Healthy women could participate only if they were pain-free on each test day (NRS score of <2/10), had no history of neck-shoulder-arm pain for longer than 8 consecutive days during the preceding year, with a pain intensity of 2 or more of 10 on the NRS, no medical consultation for neck-shoulder-arm pain during the preceding year, and no history of whiplash trauma. Additionally, healthy controls were included only if they had a score of less than 8 of 50 on the Neck Disability Index.

General exclusion criteria for all study groups were the presence of major depression or psychiatric illness; neurologic, or cardiovascular disorders; inflammatory disorders; fibromyalgia; chronic fatigue syndrome; and a history of neck or shoulder girdle surgery. All participants completed the MRI safety checklist and participants who presented con-

tra-indications for MRI were excluded. To preclude confounding factors, all participants were asked to discontinue intake of non-opioid analgesics 48 hours before study participation. In addition, participants were asked to avoid heavy physical exertion and to refrain from consuming alcohol, caffeine, and nicotine on each testing day.

Study design and procedure

This cross-sectional case-control study took place at Ghent University in cooperation with the Ghent Institute for Functional and Metabolic Imaging. The study was performed from February 2014 to September 2015 and was carried out in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of the Ghent University Hospital (EC/2013/1053) approved the research protocol. All participants were informed about the study procedures and signed an informed consent form prior to study enrolment.

First, all participants completed a survey to acquire information on demographics, and completed a series of questionnaires to obtain information on pain-related disability, pain intensity and frequency (as described below). Subsequently, experimental assessments to investigate cognitive performance and CS were performed. On a separate test day (10 +/- 7 days apart), T1-weighted MR images, diffusion-weighted MR images, and T2*-weighted gradient echo MR images of the brain were acquired.

Self-reported pain and disability measures

On each test day, participants scored current neck pain intensity on a NRS. Scores ranged from 0 to 10, with 0 reflecting 'no pain' and 10 reflecting 'the worst pain imaginable'. Patients reported their neck pain frequency in number of days per week. The Dutch Neck Disability Index was used to investigate self-reported pain-related disability levels (0-50) [42]. Higher scores on the Neck Disability Index indicate higher levels of pain-related disability. The Dutch language version of the Neck Disability Index has been proven to be valid and reliable to assess self-reported disability in patients with chronic neck pain [44, 45].

Cognitive performance

In order to obtain an objective measure of cognitive performance the Trail Making Test (TMT) was administered. This test consists of two parts, trail A and trail B. TMT part A requires mainly visuo-perceptual [46] and processing speed [47] abilities, whereas TMT part B reflects primarily working memory and secondarily task-switching ability [46]. In trail A, the participant had to draw lines connecting 25 numbers in ascending order as fast as possible, without lifting the pencil from the paper. In trail B, the participant was instructed to draw lines alternating between numbers and letters in ascending order (going from 1 to A, from A to 2, etc.). The goal of the TMT was to finish part A and part B as accurate and as quickly as possible. The time taken to complete each part of the test was used as outcome measure. Both subtests necessitate graphomotor speed, visual scanning ability, sufficient attention, and numeric sequencing [46-48]. The TMT part B further requires letter sequencing, mental double tracking, and alternation (i.e. shifting between number and letter series) [48]. The TMT has been proven to be valid for assessing cognitive deficits [46], and is a quickly administered, widely used neuropsychological test that provides information on a broad range of cognitive skills [49].

Measures of central sensitization

Distant hyperalgesia

Pressure pain thresholds (PPTs) were measured unilaterally with a digital manual algometer (Wagner Instruments, FDX, Greenwich, Connecticut) at a distant asymptomatic region (quadriceps muscle midway between the anterior superior iliac spine and the basis patellae) to evaluate secondary or distant hyperalgesia [50]. The PPTs were assessed at the most painful side [51] and in a randomized order (with research randomizer, https://www.randomizer.org). If patients experienced the same amount of neck pain at both sides, and in healthy women, PPTs were tested at the dominant handedness side. The participant was seated and pressure was gradually increased at a rate of 1 kgf/s until the participant reported the first sensation of unpleasantness. The PPT was determined as the mean of 2 consecutive (30 seconds in between) measurements. Decreased PPTs in the patient groups compared to controls at the quadriceps muscle indicate distant hyperalgesia, and are suggestive for the presence of CS. The described PPT technique has been demonstrated to be reliable [52].

Efficacy of Conditioned Pain Modulation (CPM)

Endogenous pain inhibition was investigated by applying a CPM paradigm. This paradigm relies on the "pain-inhibits-pain" mechanism in which one noxious stimulus is used as a conditioning stimulus to induce a reduction in pain perception from another test stimulus [53]. The conditioning stimulus for eliciting CPM was the cold pressor test. The PPT assessment was used as test stimulus. For the conditioning stimulus, the contralateral hand (of the PPT side) was first immersed in water maintained at room temperature (22°C) for 1 minute to standardize hand temperature [54], before immersing this hand (up to the wrist) in a refrigerated circulating bath (Versacool, Thermo Fisher Scientific, Waltham, Massachusetts) with cold water maintained at 12±1°C [55]. Participants were asked to keep their hand in the water bath for 2 minutes [54]. Meanwhile, the PPT was re-evaluated at the quadriceps muscle, approximately 45 seconds after immersing the hand (again with an interval of 30 seconds) [56]. If the participant removed the hand from the water before the end of the 2 minutes, the measurement was registered as missing. For analysis of CPM efficacy, the mean PPT measured before the cold pressor test was subtracted from the mean PPT measured during the cold pressor test. Hence, a lower CPM value reflected less efficient endogenous pain inhibition. The cold pressor test with cold water of 12°C and immersion of the hand for 2 minutes is sufficient to induce an endogenous pain inhibitory response [57].

MRI data acquisition

MR images were acquired on a 3T Siemens Magnetom TrioTim MRI scanner (Siemens, Erlangen, Germany) equipped with a 32-channel matrix head coil, at the Ghent University Hospital. First, high-resolution T1-weighted images of the brain were acquired using a three-dimensional magnetization prepared rapid acquisition gradient echo (MP-RAGE) (repetition time [TR] = 2250 ms, echo time [TE]= 4.18 ms, voxel size= 1 x 1 x 1 mm^3, field of view (FoV)= 256 x 256 mm, flip angle= 9°, 176 slices, 1 mm slice thickness, acquisition time = 5'14''). All T1-weighted anatomical scans were visually checked for overall quality and motion artefacts.

Second, DW images of the brain were acquired using single-shot echo planar imaging with a twice-refocused spin echo sequence with FoV = 240 x 240 mm, TR = 10800 ms, TE = 83 ms, and 60 contiguous sagittal slices (slice thickness = 2.5 mm; voxel size = 2.5 x 2.5 x 2.5 mm^3), and an acquisition time of 12' 36''. Diffusion sensitizing gradients were applied at a b-value of 1200 s/mm^2, along 64 noncollinear directions. Additionally, 1 set of images with no diffusion weighting (b-value= 0 s/mm^2) was acquired as a reference image.

Finally, axial T2*-weighted brain images were acquired using a T2*-weighted acquisition gradient echo with TR= 839 ms, TE= 18.60 ms, voxel size= 1 x 0.7 x 3 mm^3, FoV= 230 x 230 mm, flip angle= 20°, 3 mm slice thickness, and an acquisition time of 3' 48''. All T2*-weighted images were visually inspected by 2 expert neuroradiologists (KD, EG) to evaluate possible microhemorrhages or hemorrhagic shearing lesions related to trauma or diffuse axonal injury.

T1-weighted MRI data processing

T1-weighted anatomical scans were analyzed utilizing the FreeSurfer v5.3.0 software package (http://surfer.nmr.mgh.harvard.edu). The analyses were performed using additional computing resources from the high performance computing TIER1 cluster at the University of Ghent (http://www.ugent.be/hpc/). The FreeSurfer analysis suite was used to extract cortical thickness using an automated approach described in detail in prior publications (see Fischl 2012 [58]). Previous research has shown that this automated procedure yields accurate and reliable results [59]. Briefly, image processing included (1) removal of non-brain tissue using a hybrid watershed/surface deformation procedure (skull stripping) [60], (2) automated Talairach transformations, (3) segmentation of the subcortical WM and deep GM volumetric structures [59], (4) intensity normalization [61], (5) tessellation of the boundary between GM and WM, automated topology correction [62, 63] and (6) surface deformation along intensity gradients for optimal placement of the borders between GM, WM and cerebrospinal fluid [64, 65]. Automated parcellation of the cerebral cortex into units with respect to gyral and sulcul structures, and calculation of cortical thickness from all vertices within the cortical parcellations was performed per hemisphere using the Desikan atlas [66]. Also, estimates of total intracranial volume, and mean total thickness of the left and right hemisphere were obtained for each subject.

The T1.mgz (i.e. the FreeSurfer T1 image) and aparc+aseg.mgz (i.e. image containing ROIs constructed by the FreeSurfer pipeline) files were converted to the Neuroimaging Informatics Technology Initiative (NIfTI) format (T1.nii and aparc+aseg.nii) to be used in the further DWI analyses.

Two independent researchers (IC, RDP) visually checked the data quality of the FreeSurfer processing output including the accuracy of skull stripping, registration, segmentation, and cortical surface reconstruction. Poor data quality, such as inclusion of dura in the pial surface after skull stripping, and surface deformations, was revealed in 12 participants (controls =3, CINP =3, CWAD =6). These cortical thickness datasets were excluded from further analyses.

Cortical regions of interest

Cortical thickness was extracted from 9 ROIs from the Desikan atlas [66] reported to be involved in processing of pain and/or cognition. These ROIs were also based on our systematic review appraising the evidence for brain alterations in INP and WAD [25], on previous studies in patients with chronic pain examining GM morphology alterations [1, 5, 67], and regarding studies exploring associations between cortical thickness, and measures of cognition and pain [35, 68-70]. Specifically, the following 9 ROIs were selected from the Desikan atlas [66]: caudal anterior cingulate cortex, posterior cingulate cortex, lateral orbitofrontal cortex, superior parietal cortex, postcentral cortex, precuneus, pars orbitalis of the inferior frontal gyrus, parahippocampal cortex and supramarginal cortex (see **fig. 1** for cortical ROIs). For each ROI, cortical thickness was calculated for the right and left hemisphere separately.

DWI processing

The DWI data were analyzed and processed in ExploreDTI v4.8.6 with MATLAB [71] using the following procedure: DWI volumes were looped at a high frame rate to check for obvious artefacts in the data, such as large signal dropouts and geometric distortions. Next, we toggled between the views of the first and last acquired DW image to observe subtle system drifts. Furthermore, we inspected the images in different orthogonal views (coronal, sagittal, axial) to check for interslice and intravolume instabilities, and visualized various image maps to check for artefacts. Then, we checked the residual map for outliers, reflecting the difference between the modelled and the measured signal [29]. Next, the DW data were corrected for distortions induced by the DW gradients, artefacts due to head motion, eddy current–induced geometric distortions, and EPI distortions [72]. EPI distortions were corrected by co-registering the DW images to the T1-weighted anatomical images, which were normalized for intensity using FreeSurfer. Moreover, we performed appropriate reorientation of the encoding vectors. Next, a tensor estimation procedure was performed on the preprocessed DW data.

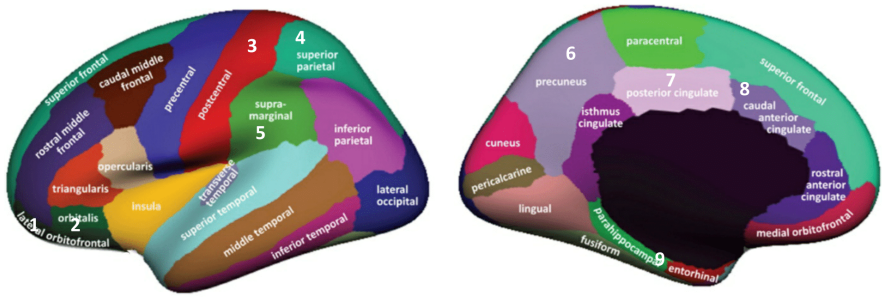


Figure 1 Lateral (left fig.) and medial (right fig.) view of the cortical parcellation of the Desikan atlas [68] displayed on an inflated template (https://surfer.nmr.mgh.harvard.edu).

Numbered regions indicate the cortical regions of interest: 1) lateral orbitofrontal cortex; 2) pars orbitalis; 3) postcentral cortex; 4) superior parietal cortex; 5) supramarginal cortex; 6) precuneus; 7) posterior cingulate cortex; 8) caudal anterior cingulate cortex; 9) parahippocampal cortex.

Each scan was visually checked for accuracy after both the motion correction and co-registration steps by 2 independent researchers. To check the accuracy of the co-registration, the preprocessed DWI was overlaid on the normalized T1-weighted anatomical image. Poor data quality was observed in 1 healthy subject because of too much head translation due to severe head motion (exceeding the size of 1 voxel), and 2 patients with CINP, 1 due to a too high percentage of outliers in the preprocessed DW images and 1 because of a general low data quality profile (ghosting, spikes). These WM microstructural data were excluded from further analyses.

Translational motions (average of axial, coronal and sagittal) did not exceed the size of 1 voxel, i.e. 2.5 x 2.5 x 2.5 mm^3 (mean +/- SE: controls: 0.85 +/- 0.03 mm; CINP patients: 0.79 +/- 0.03 mm; 0.87 +/- 0.03 mm for CWAD patients). We re-inspected the data in 3 orthogonal planes to ensure that the motion/distortion correction was performed correctly and that no additional artefacts were introduced into the data. Finally, DWI derived metrics FA, MD, AD, and RD were extracted from the preprocessed DWI data using an automated approach based on the ICBM DTI-81 WM atlas, which will be further explained in the following paragraph [73].

White matter regions of interest

The ICBM-DTI-81 WM labels atlas, developed by Mori et al. [73], was used for automated parcellation of the ROIs. This is a stereotaxic probabilistic WM atlas that fuses DTI-based WM information with the standard MNI anatomical template (ICBM-152). The WM parcellations were applied to the preprocessed images and the DWI derived metrics were calculated in each ROI, by warping the atlas template to each individual data set.

Based on our anatomical hypotheses, we selected WM atlas-labels that are mainly involved in pain or cognition or the combination thereof. The selection of these ROIs was furthermore based on previous MRI research demonstrating WM structural alterations in chronic pain or mild TBI patients, or revealing associations between WM structure, and measures of cognition and pain. Hereby, the following 10 WM regions/tracts were defined: projection fibers namely (1) the superior cerebellar peduncle [74, 75] (2) the anterior corona radiata, (3) the posterior corona radiate [11, 76-80], (4) the anterior limb of internal capsule, (5) the posterior limb of internal capsule [3]. Association fibers, namely (6) the cingulum cingulate gyrus (i.e. subgenual and retrosplenial part of the cingulum) and (7) the cingulum hippocampus (i.e. parahippocampal part of the cingulum) [3, 73] and (8) fornix and stria terminalis [81-83]. Finally, commissural fibers i.e. (9) the tapetum of the corpus callosum [2, 84] and (10) the splenium of the corpus callosum [3]. Acceleration-deceleration of the brain is believed to affect the superior cerebellar peduncles, and periventricular WM [84], e.g. tapetum of the corpus callosum [2, 85, 86]. For visualization purposes, masks of these ROIs are displayed on MD maps of the WM Mori atlas [73] in **fig. 2**. To examine WM microstructural organization, average FA, MD, AD and RD values were computed in all WM ROI for each subject for the right and left hemisphere separately.

Statistical analyses

All statistical analyses were performed with IBM SPSS Statistics 22.0. First, normality of variables was assessed with the Shapiro-Wilk test and by visual evaluation of histograms and quantile-quantile plots. Additionally, the equality of variance was examined with the Levene's test. If the assumptions of normality and equal between-group variances were met, data were analyzed with parametric tests. Otherwise, non-parametric tests were applied.

The comparability of study groups for demographics was explored with a one-way ANOVA with post-hoc pairwise comparisons using Bonferroni correction (*Family Wise Error Rate (FWER)* < 0.05), or with the Kruskal-Wallis test with post-hoc pairwise comparisons using the Mann-Whitney U test. Differences measured with the Mann-Whitney U test were only assumed significant below the significance threshold of 0.017 (Bonferroni correction: 0.05/3) to correct for the number of groups. Categorical data were analyzed with the Fisher's exact test. Group differences for cognitive performance and CS were explored with one-way ANOVA (post-hoc pairwise comparisons using Bonferroni correction, FWER < 0.05) or the Kruskal-Wallis test (post-hoc pairwise comparisons using the Mann-Whitney U test, $p < 0.017$).

An ANCOVA model was fitted, controlling for age, to examine differences in total intracranial volume and total mean thickness of the left and right hemisphere (post-hoc pairwise comparisons using Bonferroni correction, FWER < 0.05).

A MANCOVA model controlling for age was performed to determine group differences in cortical thickness of the 9 ROIs if the assumptions for performing MANCOVA were

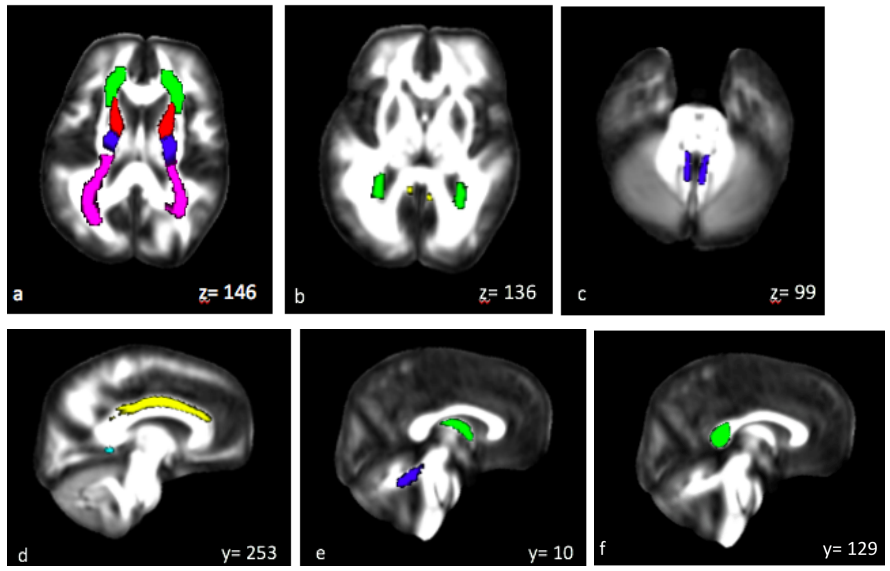


Figure 2 White matter regions of interest masks.

Regions are depicted on mean diffusivity maps of the white matter atlas of Mori et al. [73]
a= Axial view of bilateral anterior limb of internal capsule (red), bilateral posterior limb of internal capsule (blue), bilateral anterior corona radiate (green), bilateral posterior corona radiate (violet); (projection fibers)
b= Axial view of bilateral tapetum (green) (commissural fibers), and bilateral cingulum cingulate gyrus (yellow)
c= Axial view of bilateral superior cerebellar peduncles (blue); (tract in the brain stem)
d= Sagittal view of the cingulum cingulate gyrus (yellow), and the cingulum hippocampus (cyan); (association fibers)
e= Sagittal view of the fornix (green) (association fibers) and superior cerebellar peduncle (blue).
f= Splenium of the corpus callosum (green) (commissural fibers).

verified (multivariate normality in the data, homogeneity of variance between groups, absence of multicollinearity). Four MANCOVA models including age as covariate were performed investigating WM microstructure with respectively FA, MD, AD and RD of 10 WM ROIs in separate models each, as dependent variables, in patients with CWAD, CINP, and controls, as independent variables. The significance threshold was Bonferroni corrected for the number of DWI derived metrics, resulting in an adjusted *p*-value of < 0.0125 (0.05/4) for the multivariate test and for the individual WM tracts. Next, post-hoc pairwise comparison using Bonferroni correction was applied for the group comparisons.

Because there were significant effects of age on various diffusion metrics in WM regions, age was included as covariate in the MANCOVA model. For each MANCOVA model, the partial eta square (η^2) was calculated. This measure shows how much variance is explained by the independent variable (study group) and is used as the effect size for the MANCOVA model.

Finally, group-specific partial correlations (controlling for age) were conducted between cognitive performance and CS measures on one hand, and cortical thickness or WM microstructure on the other hand in cortical regions or WM tracts displaying significant group differences. To correct for multiple comparisons (for the number of clinical variables), partial correlations were deemed significant only below the 0.0125 (0.05/4) level.

Results

Demographic characteristics and self-reported pain and disability measures

The results of demographic characteristics and self-reported pain and disability measures of 102 participants (30 healthy controls, 35 CINP, 37 CWAD) are presented in **table 1**. No significant differences in demographic characteristics were found between all study groups ($p > 0.05$), except for age (healthy controls were younger compared to CINP; $p = 0.010$). Furthermore, both patient groups were comparable in use of medications, neck pain duration, and frequency of neck pain complaints ($p > 0.05$). In contrast, higher neck pain intensity at the MRI test day ($p < 0.001$), and higher pain-related disability were reported by patients with CWAD compared to CINP ($p = 0.001$).

Control analyses

The MANCOVA with age and handedness as covariates, revealed no significant main effect of handedness on regional cortical thickness ($p = 0.284$) or WM microstructure ($p = 0.349$ for FA; $p = 0.215$ for MD; $p = 0.170$ for AD; $p = 0.094$ for RD). Therefore, the cortical thickness and WM microstructure results of the left- and right-handed women were analyzed together. The ANCOVA controlling for age showed no significant group differences for total intracranial volume ($p = 0.137$), and total mean thickness of the left ($p = 0.563$) and right ($p = 0.404$) hemisphere.

The assumptions for all applied models were verified.

Group differences in cognitive performance and central sensitization

As shown in **table 1**, the completion time of TMT part A ($p < 0.001$) and part B ($p = 0.002$) was significantly longer in the CWAD group compared to healthy controls, denoting worse performance in CWAD patients. In addition, the time needed to perform TMT part A ($p < 0.001$) was significantly longer in patients with CWAD compared to CINP.

Table 1 Demographic characteristics, self-reported pain and disability measures, cognitive performance, distant hyperalgesia and conditioned pain modulation efficacy in patients with CWAD, CINP and healthy controls.

			Median	IQR	Range (min-max)	Test statistic (*P*-value)	*P*-value post-hoc
Demographic characteristics	**Age (y)**[a]	***HCON***	25	22-44	18-62	8.020 (**0.018**)	**0.010**[d]
		CINP	36	29-50	19-62		0.017[e]
		CWAD	38	25 – 47	21-59		0,769[f]
	Body mass Index (kg/m²)[a, †]	***HCON***	20.94	20.13-22.90	17.99 – 26.75	3.462 (0.177)	0.069[d]
		CINP	23.05	20.12-25.62	18.34-29.07		0.438[e]
		CWAD	22.31	19.60-23.88	16.65 -31.46		0.242[f]
			Frequencies				
	Education level, n (%)[c] No degree; lower secon.; Higher second.; Higher edu.	***HCON***	0 (0); 1 (3.3); 7 (23.3); 21 (70)			2.172 (1.000)	NA
		CINP	0 (0); 1 (2.9); 8 (22.9); 23 (65.7)				
		CWAD	1 (2.7); 1 (2.7); 9 (24.3); 25 (67.6)				
	Smoker, n (%)[c] Smoker; former smoker; non-smoker	***HCON***	1 (3.3); 3 (10); 25 (83.3)			7.908 (0.066)	NA
		CINP	0 (0); 9 (25.7); 18 (51.4)				
		CWAD	4 (10.8); 10 (27); 23 (62.2)				
	Handedness, n (%)[c] (LH; RH)	***HCON***	5 (17.2) ; 24 (82.8)			2.241 (0.344)	NA
		CINP	2 (5.7); 33 (94.3)				
		CWAD	3 (8.8); 31 (91.2)				
***Demographic characteristics*: medication use**	**Analgesics – antipyretics n (%)**[c]	***HCON***	0 (0)			10.962 (**0.003**)	0.069[f]
		CINP	3 (8.6)				
		CWAD	10 (27)				
	Narcotic analgesics n (%)[c]	***HCON***	0 (0)			2.261 (0.330)	NA
		CINP	0 (0)				
		CWAD	2 (5.4)				
	Benzodiazepines n (%)[c]	***HCON***	1 (3.3)			5.768 (0.053)	NA
		CINP	1 (2.9)				
		CWAD	7 (18.9)				
	Antidepressants (%)[c]	***HCON***	0 (0)			2.479 (0.316)	NA
		CINP	3 (8.6)				
		CWAD	2 (5.4)				
Self-reported pain measures	**Neck pain duration (months)**[a]	***HCON***	NA	NA	NA	531.000 (0.882)	*NA*
		CINP	60	22-120	4-288		
		CWAD	60	30-120	3-444		
	Days/week neck pain?[a]	***HCON***	NA	NA	NA	228.000 (0.035)	*NA*
		CINP	5	3-7	3-7		
		CWAD	7	5-7	2-7		
	Current neck pain intensity (VNRS/10)_C[a, †]	***HCON***	0	0 – 0	0 – 0	35.582 (**<0.001**)	***<0.001***[d]
		CINP	4	2-7	0-8		***<0.001***[e]
		CWAD	7	5-7	0-10		*0.026*[f]
	Current neck pain intensity (VNRS/10)_M[a, †]	***HCON***	0	0 – 0	0 – 0	71.625 (**<0.001**)	***< 0.001***[d]
		CINP	3	1-5	0-6		***< 0.001***[e]
		CWAD	6	4-8	1-10		***< 0.001***[f]
Self-reported disability	**Neck Disability Index (/50)**[a, †]	***HCON***	2	1-4	0-6	65.109 (**< 0.001**)	***< 0.001***[d]
		CINP	16	12-20	10-27		***< 0.001***[e]
		CWAD	22	22	10-37		***0.001***[f]
Objective cognitive performance	**TMT part A (sec)**[a, †]	***HCON***	18.61	18.61	10.03-29.75	19.247 (**<0.001**)	*0.339*[d]
		CINP	19.37	19.37	11.56-28.40		***<0.001***[e]
		CWAD	27.09	27.09	15.06-81.00		***<0.001***[f]
	TMT part B (sec)[a, †]	***HCON***	34.37	27.25-45.89	21.44-128.00	11.727 (**0.003**)	*0.121*[d]
		CINP	38.36	31.45-44.36	26.60-148.00		***0.002***[e]
		CWAD	46.00	36.71-77.50	25.86-251.00		*0.017*[f]
Distant HA	**PPT quadriceps (kgf)**[b]	***HCON***	5.087	1.633	2.940-8.790	6.587 (**0.002**)	*0.382*[d]
		CINP	4.383	1.945	1.700-9.720		***0.002***[e]
		CWAD	3.432	1.840	0.300-7.720		*0.092*[f]
CPM efficacy	**CPM quadriceps** (PPT quadriceps during CPT minus PPT quadriceps before CPT)[b]	***HCON***	1.146	0.703	-0.140-3.000	5.698 (**0.005**)	*1.000*[d]
		CINP	0.937	0.929	-0.590-3.290		***0.005***[e]
		CWAD	0.427	0.853	-1.050-1.870		*0.061*[f]

[a]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and for post-hoc pairwise comparisons the Mann-Whitney U test. To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.017 level (Bonferonni correction: 0.05/3). [b]= Data which were assumed to be normally distributed and were variances were equally distributed across groups were analyzed with one-way ANOVA (F-test) and post-hoc pairwise comparisons were applied using Bonferroni correction ($p<0.05$). [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. [†] Variances were not equally distributed across groups, Levene's test $p<0.05$, [d]= *p*-value for significant differences between CON-CINP, [e]= *p*-value for significant differences between CON-CWAD, [f]= *p*-value for significant differences between CINP-CWAD. Abbreviations: y= years. HCON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain. VNRS= verbal numeric rating scale. No degr= no degree, Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, HA= hyperalgesia, CPM= conditioned pain modulation, CPT= cold pressor test, TMT= trail making test, kgf= kilogram force, PPT= pressure pain thresholds, VNRS= verbal numeric rating scale, IQR= interquartile range.

Data of 102 subjects were analysed (30 HCON, 35 CINP, 37 CWAD) for the demographic variables, the self-reported pain and disability measures. Data of 97 subjects were analysed (28 HCON, 35 CINP, 34 CWAD) for the current neck pain intensity at the clinical test day, TMT, PPT and CPM efficacy.

Decreased PPTs were demonstrated at the quadriceps muscle, reflecting distant hyperalgesia in patients with CWAD compared to healthy controls ($p = 0.002$). The CPM value, measured at the quadriceps muscle, was significantly lower in CWAD patients compared to controls ($p = 0.005$), indicating diminished endogenous pain inhibition in patients with CWAD (**table 1**).

Group differences in cortical thickness

The MANCOVA model examining cortical thickness in 9 ROIs between individuals with CWAD, CINP, and healthy controls, including age as covariate, demonstrated significant differences for cortical thickness ($p = 0.015$, $\eta^2 = 0.312$), based on the multivariate test (**supplementary table A**). Only the left precuneus showed significant group differences in cortical thickness ($p = 0.037$). Post-hoc pairwise comparisons between study groups using Bonferroni correction revealed significant decreased cortical thickness in the left precuneus ($p = 0.032$) (**fig. 3, supplementary table A**) in patients with CWAD compared to CINP.

Group differences in white matter microstructure

As can be seen in **supplementary table B**, four MANCOVA models with age as covariate investigating WM microstructure with respectively FA, MD, AD, and RD of 10 WM tracts in separate models in patients with CWAD, CINP, and controls, showed significant differences for FA ($p = 0.010$, $\eta^2 = 0.293$), MD ($p = 0.007$, $\eta^2 = 0.274$), and RD ($p = 0.007$, $\eta^2 = 0.297$) based on the multivariate tests. The MANCOVA of AD could not detect significant differences between study groups ($p = 0.499$), hence regional WM differences in AD were not further analysed.

The following WM tracts did retain significance after correcting for multiple comparisons ($p < 0.0125$ (0.05/4)): left cingulum hippocampus ($p = 0.002$ for FA, $p = 0.004$ for MD, $p = 0.002$ for RD) and left tapetum ($p = 0.003$ for FA, $p = 0.005$ for MD, $p = 0.004$ for RD) (**supplementary table B**). Subsequent, post-hoc pairwise comparisons between study groups, using Bonferroni correction, revealed significantly decreased FA ($p = 0.007$ and $p = 0.013$), increased MD ($p = 0.010$ and $p = 0.025$), and increased RD ($p = 0.009$ and $p = 0.020$) in the left tapetum in CWAD patients compared to controls and CINP patients respectively (**fig. 4, supplementary table B**). Also, significantly decreased FA ($p = 0.002$), increased MD ($p = 0.004$), and increased RD ($p = 0.001$) in the left cingulum hippocampus were found in CWAD patients compared to CINP patients (**fig. 4, supplementary table B**).

Results of T2*-weighted brain imaging analyses

Based on detailed visual inspection of the axial T2*-weighted brain images, no hemorrhagic shearing lesions or microhemorrhages related to trauma or diffuse axonal injury were detected. In one CWAD patient a small a-specific T2* hypointensity left parietal without clinical relevance was observed. Furthermore, in one CINP patient the neuroradiologists

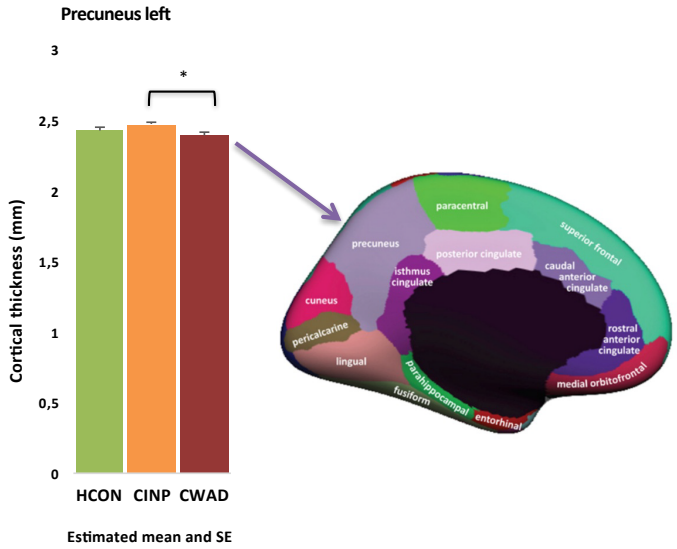


Figure 3 Significant differences in cortical thickness of grey matter ROIs between patients with CWAD (n= 31), CINP (n= 32) and healthy controls (n= 26).

Abbreviations: HCON= healthy pain-free controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, SE= standard error, ROIs= regions of interest. Data were analyzed using MANCOVA with age as covariate and post-hoc pairwise comparisons were applied using Bonferroni correction. *= p=0.032

detected a small round T2* hypointensity in the left thalamus and left globus pallidus suggesting microhemorrhages related to hypertension.

Associations between cortical thickness alterations, and cognitive performance and central sensitization

Associations (partial correlations corrected for age) between cognitive performance and CS, and cortical thickness were investigated only in ROIs showing significant group differences (**table 2**). The time to complete TMT part B was negatively correlated with cortical thickness of the left precuneus ($r = -0.520$, $p = 0.005$) within the CINP group (n = 27). Specifically, worse performance on the TMT part B coincided with decreased cortical thickness in the left precuneus in patients with CINP, however worse TMT performance could not be revealed in CINP patients compared to healthy controls. In the CWAD (n = 26), and control group (n = 26), no significant correlations could be found ($p > 0.017$).

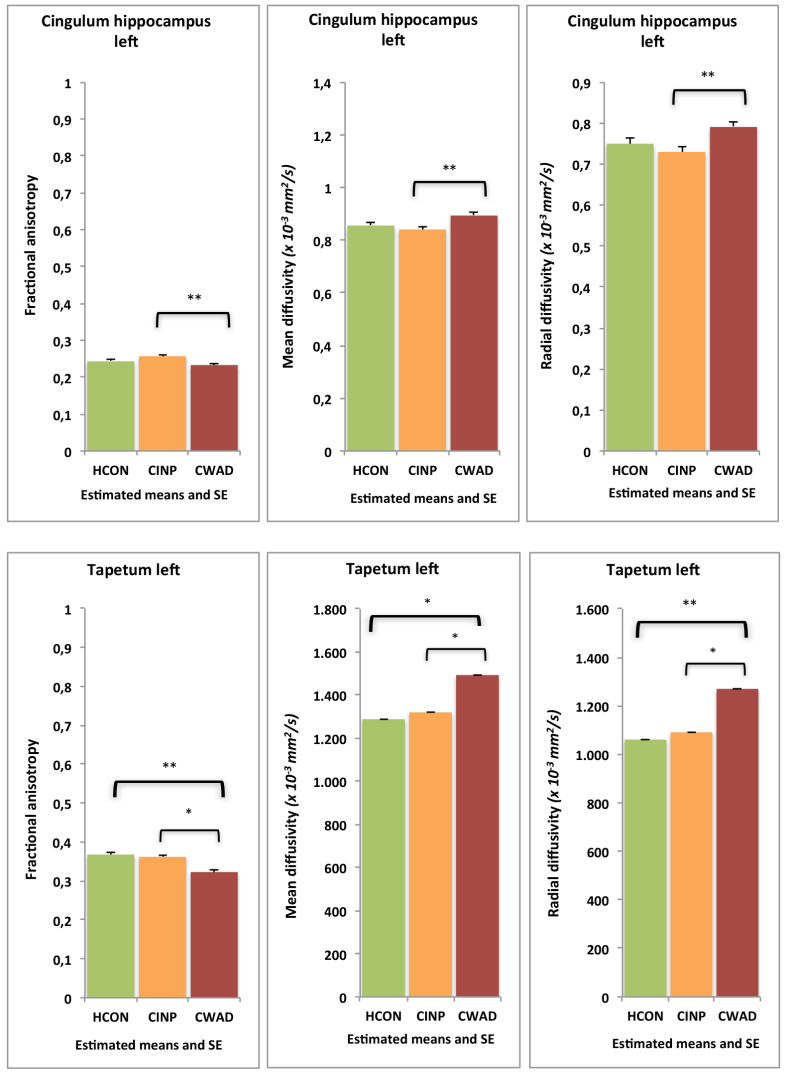


Figure 4 Significant differences in DTI-derived metrics of white matter ROIs between CWAD patients (n= 37), CINP patients (n= 35) and healthy women (n= 30).

Abbreviations: HCON= healthy pain-free controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, SE= standard error, DTI= diffusion tensor imaging, ROIs= regions of interest. Data were analyzed using MANCOVA with age as covariate (p<0.0125 (0.05/4)) and post-hoc pairwise comparisons were applied using Bonferroni correction. *= p<0.05, **= p<0.01.

Table 2 Partial correlations (controlling for age) between **grey matter ROIs with significant group differences,** and **experimental measures of pain and cognition** in patients with CINP and CWAD.

GM ROI cortical thickness (mm)	visuo-perceptual and processing speed abilities	working memory and task-switching ability	Distant hyperalgesia	CPM efficacy
	TMT A	TMT B	PPT quadriceps	CPM quadriceps
CINP (n= 27)				
Precuneus left	-0,164	**-0,520**	-0,241	0,030
	0,414	*0,005*	*0,208*	*0,886*
CWAD (n= 26)				
Precuneus left	-0,022	-0,107	0,154	0,411
	0,914	*0,596*	*0,433*	*0,051*

To correct for multiple comparisons, correlations significant at a statistical threshold level of P< 0.0125 level (0.05/4) (2-tailed) were deemed significant, and are presented in bold and in green. P-values are presented below the correlation coefficient. In healthy pain-free controls, no significant correlations could be revealed (data not presented). Abbreviations: CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, TMT= trail making test, PPT= pressure pain thresholds, CPM= conditioned pain modulation, ROIs= regions of interest.

Associations between white matter microstructural alterations, and cognitive performance and central sensitization

As presented in **table 3**, associations (partial correlations corrected for age) between WM microstructure (only FA, MD, RD), and cognitive performance and CS measures were investigated only in WM tracts demonstrating significant group differences. In the CWAD group (n = 34), efficacy of CPM was negatively correlated with MD (r = - 0.478, p = 0.010) and RD (r = - 0.477, p = 0.010) in the left tapetum. In other words, decreased efficacy of CPM was moderately correlated with increased MD and RD in the left tapetum in CWAD patients. In the CINP (n = 33) and control group (n = 28), no significant correlations could be revealed (p-value > 0.0125 (0.05/4)).

Table 3 Partial correlations (controlling for age) between **DTI-derived metrics in WM ROIs with significant group differences,** and **measures of cognition and central sensitization** in patients with CINP and CWAD.

WM ROI	DTI-derived metric	visuo-perceptual and processing speed abilities	working memory and task-switching ability	Distant hyperalgesia	CPM efficacy
		TMT A	TMT B	PPT quadriceps	CPM quadriceps
CINP (n= 33)					
CgH left	**FA**	-0,113	-0,212	0,068	-0,038
		0,552	*0,260*	*0,712*	*0,848*
	MD	0,330	0,356	-0,093	-0,082
		0,075	*0,053*	*0,612*	*0,679*
	RD	0,304	0,364	-0,103	-0,042
		0,102	*0,048*	*0,576*	*0,831*
TAP left	**FA**	-0,376	-0,330	-0,093	0,068
		0,040	*0,075*	*0,614*	*0,730*
	MD	0,001	0,197	0,059	-0,076
		0,998	*0,297*	*0,748*	*0,700*
	RD	0,078	0,245	0,079	-0,081
		0,684	*0,193*	*0,668*	*0,682*
CWAD (n= 34)					
CgH left	**FA**	-0,198	-0,025	0,213	-0,021
		0,278	*0,891*	*0,235*	*0,915*
	MD	-0,006	-0,077	-0,142	0,418
		0,974	*0,677*	*0,432*	*0,027*
	RD	0,022	-0,065	-0,15	0,379
		0,903	*0,725*	*0,405*	*0,047*
TAP left	**FA**	-0,295	-0,240	0,197	0,397
		0,102	*0,187*	*0,272*	*0,037*
	MD	0,332	0,287	-0,152	**-0,478**
		0,063	*0,112*	*0,397*	*0,010*
	RD	0,342	0,290	-0,171	**-0,477**
		0,055	*0,107*	*0,340*	*0,010*

Correlations significant at a statistical threshold level of $P< 0.0125$ level (0.05/4) (2-tailed) were deemed significant, and are presented in bold and in green. *P*-values are presented below the correlation coefficient. In healthy pain-free controls, no significant correlations could be revealed (data is not presented). Abbreviations: CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, DTI= diffusion tensor imaging, TMT= trail making test, PPT= pressure pain thresholds, CPM= conditioned pain modulation, WM= white matter, ROI= region of interest, CgH= Cingulum hippocampus, TAP= Tapetum, FA= fractional anisotropy, MD= mean diffusivity, RD= radial diffusivity.

Discussion

The present study has demonstrated cortical thinning in the left precuneus, a region crucially involved in cognitive functioning, in patients with CWAD compared to CINP. Furthermore, abnormalities in WM microstructure encompassing decreased FA coinciding with increased MD and RD in the left cingulum hippocampus were revealed in patients with CWAD compared to CINP, and in the left tapetum in patients with CWAD compared to CINP and healthy controls. Interestingly, brain microhemorrhages related to trauma were not observed. This is the first study revealing alterations in cortical thickness and WM microstructure in women with CWAD compared to CINP or healthy women, indicating a potential association between these brain alterations and the signs and symptoms induced by the traumatic event in patients with CWAD. Moreover, decreased CPM efficacy (denoting CS) was associated with the extent of WM deficits in the left tapetum in CWAD patients. The latter yields novel evidence for underlying microstructural neural correlates of disturbed endogenous pain inhibition.

Group differences in cognitive performance and central sensitization

CWAD patients displayed worse processing speed abilities compared to CINP patients and controls based on the results of the TMT part A. Decreased working memory span was revealed in the CWAD group compared to controls, utilizing the TMT part B. Consistent with these findings, 2 recent studies revealed worse cognitive performance in CWAD patients compared to healthy volunteers, specifically on domains of sustained attention and working memory evaluated with computer-based cognitive tasks [34, 87]. Hence, our study highlights the importance of evaluating cognitive performance in patients with CWAD in clinical practice.

Furthermore, CWAD patients showed decreased PPTs at the quadriceps muscle compared to healthy controls reflecting distant hyperalgesia. This indicates significant indirect signs of CS and thus enhanced central pain sensitivity in CWAD. In accordance with our expectations, distant hyperalgesia was not demonstrated in CINP patients. Likewise, Scott and colleagues reported decreased PPTs at distant regions in CWAD but not in CINP patients [23]. Furthermore, decreased CPM efficacy was only observed in the CWAD group, implying disturbed endogenous pain inhibitory mechanisms in CWAD but not in CINP patients. Consistent with these findings, reduced endogenous pain inhibition has previously been reported in CWAD [88, 89] but could not be found in patients with CINP [90]. The lack of signs for CS in CINP patients is furthermore in line with the results of a recent systematic review concluding that CS is not a characteristic feature of CINP [22]. Our results add evidence to support that CS is present, at a group level, in CWAD and usually not in CINP patients, which further elucidates difference in underlying mechanisms.

Group differences in cortical thickness

The cortical thinning in the left precuneus in CWAD supports our hypothesis of decreased regional cortical thickness in patients with traumatic compared to non-traumatic chronic neck pain. The observed cortical thinning in the precuneus in CWAD compared to CINP patients has a valuable meaning because it demonstrates macrostructural differences between both patient groups. Moreover, this region plays a crucial role in a wide range of cognitive and mental processes, and is involved in neurodegenerative processes [91, 92]. The precuneus is part of the structural core of the brain [93], and is a core hub (i.e. highly interconnected nodes) of the default mode network [94]. The latter network is a constellation of brain regions involved in self-referential mental activity, emotional processing, and recollection of prior experiences, and is deactivated during externally focused tasks [95]. It is reported that damage to network hub regions connecting different subnetworks, such as the precuneus, causes the largest disturbances in network organization [96]. Also, functional MRI studies have demonstrated altered resting-state functional connectivity in the precuneus in patients with chronic musculoskeletal pain [97], and in mild TBI patients [98]. Furthermore, Baliki et al. [99] have demonstrated reorganization of the default mode network dynamics in different chronic pain conditions including alterations in precuneus connectivity. Interestingly, Kucyi et al. [100] found that functional connectivity fluctuations between the default mode network and periaqueductal grey dynamically tracked spontaneous attention away from pain in healthy people.

Nevertheless, opposite to our hypothesis, we could not detect significant correlations between cortical thinning in the precuneus and cognitive performance or experimental CS measures in CWAD patients. Also, in contrast to our hypothesis and to the results of previous studies in patients with chronic musculoskeletal pain [5, 101] and mild TBI [9], differences in cortical thickness between CWAD patients and healthy controls could not be demonstrated. Yet, the cortical thickness MANCOVA model comprising all ROIs showed a partial eta squared of 0.31, which means that 31% of the variance in cortical thickness in the 9 ROIs can be explained by study group, which corresponds with a large effect size.

Group differences in white matter microstructure

Results showed a consistent pattern of decreased FA coinciding with increased MD and RD in the left cingulum hippocampus, and in the left tapetum in patients with CWAD compared to healthy controls (tapetum) or compared to CINP patients (cingulum and tapetum). In contrast, AD values were not different between all study groups.

The observed WM microstructural differences between CWAD patients compared to healthy controls were in line with our hypothesis. In addition, differences in WM microstructure were revealed between CINP and CWAD patients in the left cingulum hippocampus and the left tapetum. The tapetum of the corpus callosum is periventricular WM, which is believed to be affected by traumatic acceleration-deceleration of the brain [85, 86]. To date, limited studies are available on WM abnormalities in the tapetum as separate region. The tapetum is the temporal component of the corpus callosum [73] formed by decussating fibers in the splenium that arch over the atrium of the lateral ventricle and course inferiorly in the posterior and temporal horns of this ventricle [85]. In contrast, more evidence exists for WM deficits in the corpus callosum (genu, rostrum, body, splenium, tapetum) in chronic musculoskeletal pain patients [3] and mild TBI patients [102].

The revealed pattern of WM microstructural deficits encompassing decreased FA, increased MD and increased RD in CWAD is consistent with results in other chronic musculoskeletal pain conditions. For example, Lieberman et al [3] observed decreased FA and increased RD in the left cingulum hippocampus in chronic musculoskeletal pain patients compared to healthy volunteers. In patients with complex regional pain syndrome decreased FA in the left cingulum bundle has also been demonstrated [103]. Furthermore, the observed WM abnormalities are in accordance with findings of increased MD in the cingulum in mild TBI patients compared to healthy persons [104]. In literature, studies tend to show decreased FA associated with numerous neurological and neurodegenerative diseases [105, 106].

Additionally, WM microstructural abnormalities were found in the cingulum hippocampus in CWAD compared to CINP patients but not compared to healthy persons. The cingulum bundle is a prominent WM tract extending longitudinally above the corpus callosum [107], and carrying information from the cingulate gyrus to the hippocampus. Specifically, the cingulum contains many afferent and efferent fibers associated with the cingulate cortices [108-110]. These fibers include connections with the thalamus, dorsolateral prefrontal cortex, and insula [108]. Other cingulum fibers are connected to the parahippocampal cortices, the inferior component of the hippocampal formation, and amygdala [108, 110].

We can very carefully suggest that our findings are indicative of WM microstructural abnormalities in the left tapetum and the left cingulum hippocampus in the CWAD group, maybe to some extent reflecting WM demyelination evidenced by the unchanged AD together with increased RD [111-113]. Interestingly, in mild TBI patients research has demonstrated that long associative WM tracts such as the cingulum bundle are frequently affected [114, 115]. As such, the underlying mechanisms of the revealed WM deficits in CWAD patients could be associated with the traumatic whiplash injury, which perhaps induced a cascade of events eventually leading to WM microstructural deficits. Noteworthy, FA differences in WM have been shown to predict transition to chronic pain in subacute low back pain patients [6]. Nevertheless, based on visual inspection of the T2*-weighted brain images, microhemorrhages related to trauma or diffuse axonal injury were not observed in patients with CWAD. In patients with mild TBI, previous studies could detect subtle microhemorrhagic lesions suggesting differences in underlying pathophysiological mechanisms between patients with chronic WAD and mild TBI [116]. However, in future research we should include Susceptibility Weighted Imaging or Quantitative Susceptibility Mapping to detect possible microhemorrhages related to trauma in chronic WAD patients.

Decreased cortical thickness in the precuneus: associations with cognitive performance and central sensitization

In CINP patients, decreased precuneus thickness coincided with worse performance on the TMT part B, however decreased cortical thickness and worse cognitive performance could not be revealed in CINP patients compared to controls. The observed association between working memory capacity and cortical thickness in CINP patients is in line with studies reporting associations between regional GM morphology and working memory capacity or other features of cognition in patients with fibromyalgia [36], patients with complex regional pain syndrome [117], and in mild TBI patients [118].

Microstructural white matter abnormalities in the cingulum hippocampus and tapetum: associations with cognitive performance and central sensitization

Consistent with our hypothesis, deficits in WM microstructure in the left tapetum, encompassing increased MD and RD, were associated with decreased CPM efficacy (denoting CS) in the CWAD group. However, associations between cognitive performance or CS, and the observed WM alterations in the cingulum hippocampus could not be detected. Also, no associations were demonstrated between cognitive performance or distant hyperalgesia, and WM microstructural abnormalities in CWAD patients, which was in contrast with our expectations. Interestingly, we provide novel evidence for associations between microstructural WM deficits and decreased CPM efficacy in CWAD. Recently, a longitudinal MRI study uncovered microstructural WM vulnerabilities to develop chronic back pain [6]. Also, moderate evidence exists to support that higher pain intensity is associated with FA alterations in WM tracts involved in pain and cognition [35].

Clinical implications

Our findings support a role of WM microstructural abnormalities in the left tapetum in the observed dysfunctional CPM response in CWAD. This yields innovative evidence for underlying WM microstructural correlates of disturbed endogenous pain inhibition in CWAD but not in CINP patients. Accordingly, these results emphasize the role of WM abnormalities in patients with CWAD compared to CINP and maybe reflect one piece of the puzzle underlying the observed clinical differences between both neck pain conditions with possibly a role of the traumatic injury mediating the structural brain differences.

Our results furthermore indicate that chronic pain in CWAD patients should be interpreted, at least in part, as a result of structural plasticity of the central nervous system, associated with alterations in cortical thickness and WM microstructure in regions involved in various aspects of pain and cognitive processing. Accordingly, it can be recommended that therapy approaches for CWAD should address the brain and take into account neuroplasticity of the central nervous system. As such, clinicians should take into account the observed structural brain differences when treating patients with CINP and CWAD.

Strengths, limitations and recommendations for further research

Several strengths can be outlined. This is the first study examining alterations in cortical thickness and WM microstructure, and their relationships with cognitive performance and CS in patients with a traumatic origin of pain (CWAD) compared to patients with non-traumatic CINP and healthy controls. This research is important because it unraveled differences between both pain conditions, and provided evidence for CS and structural brain alterations as underlying pathophysiological mechanisms in CWAD. Another strength is the evaluation of alterations in both GM macrostructure (cortical thickness) and WM microstructure (FA, MD, AD, RD).

The following limitations have to be taken into account when interpreting our results. Metrics derived from DWI data using the tensor model are indirect measures that relate to but do not directly quantify tissue features and are influenced by various methodological and biological factors [105]. These metrics cannot disentangle the individual microscopic contributions at the voxel level and therefore should be interpreted with caution. It remains unclear whether decreased FA is due to changes in membrane permeability, organelles, axon thickness, fiber density, or degree of myelination [105]. It has been suggested that alterations in RD combined with unchanged AD reflect demyelination [112], but the interpretation of these metrics has been a topic of controversy [119].

A second limitation of DTI analyses is the inability of the tensor model to adequately characterize diffusion in regions of complex fiber architecture. Single-shell data reconstructed with the diffusion tensor model assumes a single straight fiber orientation within each voxel and is inadequate to model more than one fiber orientation per voxel. In the brain however voxels often contain fiber populations with more than one dominant orientation, such as crossing fibers [105]. Therefore, advanced models based on the high angular resolution DWI acquisition strategy to provide more robust estimates of the fiber orientation are recommended for further research in CINP and CWAD.

A final limitation pertains to the cross-sectional nature of this study implying that no conclusions can be drawn on the causality of the observed associations. To test causal inference, future longitudinal studies are necessary.

Further research investigating associations between abnormalities in WM microstructure in CWAD, and other experimental features of CS such as temporal summation of second pain could add valuable insights into the brain structural correlates of CS. Finally, it could be recommended to disentangle possible functional brain alterations using (resting-state) functional MRI techniques and perform network analyses in patients with CWAD and CINP compared to healthy controls.

Conclusion

In conclusion, cortical thinning in the left precuneus, a core hub of the default mode network and part of the structural brain core, was found in women with CWAD compared to CINP. Additionally, abnormalities in WM microstructure were revealed in 2 WM tracts carrying information between regions involved in affective-cognitive dimensions of pain processing and cognition in women with CWAD compared to CINP or healthy women. This study provided novel evidence for associations between dysfunctional CPM and the degree of WM deficits in the left tapetum in patients with CWAD. This yields innovative evidence for underlying WM microstructural correlates of CS and in particular of disturbed endogenous pain inhibition in CWAD. Accordingly, these results emphasize the role of structural brain alterations in patients with CWAD compared to CINP.

Acknowledgements

The authors gratefully acknowledge the study participants, without whom this study could have never been completed successfully. For its financial support, the authors' gratitude goes out to the Special Research Fund of Ghent University (BOF-Ghent), which made this experimental research possible. We acknowledge the University of Ghent for the possibility to use the additional computing resources from the high performance computing (HPC) TIER1 cluster (http://www.ugent.be/hpc/) for performing the FreeSurfer analyses. Finally, we acknowledge Stephanie Bogaert for her radiological assistance with the T2*-weighted MRI analyses.

Supplementary table A Differences in cortical thickness of ROIs involved in processing of cognition and pain in CWAD patients, CINP patients and healthy women.

Multivariate tests	Wilks' λ Value	F-value*	*P*-value	Partial eta square (η^2)
ROIs (left and right hemisphere)				
Caudal ACC, PCC, lateral orbitofrontal cortex, pars orbitalis, postcentral cortex, precuneus, superior parietal, supramarginal, parahippocampal cortex	0.474	1.710	**0.015**	0.312

*= Wilks' Lambda exact statistic, age was included as covariate in the multivariate analysis of covariance (MANCOVA). Significant *p*-values are presented in bold.

Estimated means					Tests of between-Subjects Effects		Pairwise comparisons Adjustment for multiple comparisons: Bonferroni.				
	Mean*	Std. Error	95% Confidence Interval		F-value	*P*-value		Estimated Mean Difference	*P*-value	95% Confidence Interval for Difference	
			Lower Bound	Upper Bound						Lower Bound	Upper Bound
Precuneus cortical thickness (mm) left hemisphere											
HCON	2.427	0.022	2.384	2.470	3.442	**0.037**	**HCON-CINP**	-0.041	0.509	-0.112	0.031
CINP	2.468	0.019	2.430	2.506			**HCON-CWAD**	0.031	0.894	-0.041	0.103
CWAD	2.396	0.020	2.358	2.435			**CINP-CWAD**	0.071	**0.032**	0.005	0.138

*Covariates appearing in the model are evaluated at the following values: age = 35.71
HCON n= 26; CINP n= 32; CWAD n=31.
Abbreviations: HCON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, ROIs= regions of interest, ACC= anterior cingulate cortex, PCC= posterior cingulate cortex.

Supplementary table B Significant differences in DTI-derived metrics of white matter ROIs in CWAD patients, CINP patients and healthy women.

Multivariate tests	Diffusion-derived metric	Wilks' λ Value	F-value[γ]	*P*-value*	Partial eta square (η^2)
ROIs (left and right hemisphere)					
SCP ALIC, PLIC ACR, PCR CgG H, CgC gyrus FX and Stria terminalis SCC, TAP	**FA**	0.500	1.744	**0.010**	0.293
	MD	0.527	1.822	**0.007**	0.274
	AD	0.656	0.987	0.499	0.190
	RD	0.494	1.782	**0.007**	0.297

[γ] = Wilks' Lambda exact statistic, age was included as covariate in the multivariate analysis of covariance (MANCOVA). Significant *P*-values are presented in bold.
**P*-values were only deemed significant below the 0.0125 level (0.05/4) to correct for multiple comparisons.

		Estimated means				Tests of between-Subjects Effects		Pairwise comparisons Adjustment for multiple comparisons: Bonferroni.			
		Mean*	Std. Error	95% Confidence Interval		F-value	*P*-value*		Estimated Mean Difference	*P*-value	95% Confidence Interval for Difference
				Lower Bound	Upper Bound						Lower Bound / Upper Bound
Fractional anisotropy											
CgH left	**HCON**	0.243	0.005	0.233	0.254	6.434	**0.002**	**HCON-CINP**	-0.013	0.213	-0.031 / 0.004
	CINP	0.257	0.005	0.247	0.266			**HCON-CWAD**	0.011	0.397	-0.007 / 0.028
	CWAD	0.233	0.005	0.223	0.242			**CINP-CWAD**	0.024	**0.002**	0.008 / 0.040
TAP left	**HCON**	0.368	0.011	0.347	0.388	6.281	**0.003**	**HCON-CINP**	0.005	1.000	-0.030 / 0.040
	CINP	0.362	0.010	0.343	0.382			**HCON-CWAD**	0.044	**0.007**	0.010 / 0.079
	CWAD	0.323	0.009	0.305	0.342			**CINP-CWAD**	0.039	**0.013**	0.007 / 0.071
Mean diffusivity (x 10^{-3} mm^2/s)											
CgH left	**HCON**	0.856	0.013	0.831	0.881	5.725	**0.004**	**HCON-CINP**	0.014	1.000	-0.028 / 0.057
	CINP	0.841	0.012	0.818	0.864			**HCON-CWAD**	-0.038	0.082	-0.080 / 0.003
	CWAD	0.894	0.011	0.872	0.916			**CINP-CWAD**	-0.053	**0.004**	-0.092 / -0.014
TAP left	**HCON**	1.288	0.051	1.187	1.389	5.605	**0.005**	**HCON-CINP**	-0.032	1.000	-0.201 / 0.137
	CINP	1.320	0.046	1.228	1.412			**HCON-CWAD**	-0.205	**0.010**	-0.371 / -0.038
	CWAD	1.493	0.045	1.404	1.582			**CINP-CWAD**	-0.173	**0.025**	-0.329 / -0.016
Radial diffusivity (x 10^{-3} mm^2/s)											
CgH left	**HCON**	0.750	0.013	0.724	0.776	6.811	**0.002**	**HCON-CINP**	0.020	0.833	-0.024 / 0.064
	CINP	0.730	0.012	0.706	0.754			**HCON-CWAD**	-0.041	0.072	-0.084 / 0.003
	CWAD	0.791	0.012	0.768	0.814			**CINP-CWAD**	-0.061	**0.001**	-0.102 / -0.020
TAP left	**HCON**	1.061	0.051	0.960	1.162	5.861	**0.004**	**HCON-CINP**	-0.030	1.000	-0.200 / 0.140
	CINP	1.091	0.047	0.999	1.184			**HCON-CWAD**	-0.209	**0.009**	-0.376 / -0.042
	CWAD	1.270	0.045	1.181	1.360			**CINP-CWAD**	-0.179	**0.020**	-0.336 / -0.021

*Covariates appearing in the model are evaluated at the following values: age = 36.08
HCON n= 30; CINP n= 35; CWAD n=37. **P*-values were only deemed significant below the 0.0125 level (0.05/4) to correct for multiple comparisons.
Abbreviations: HCON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash associated disorders, ROIs= regions of interest, CgH= Cingulum hippocampus, TAP= Tapetum, FX= fornix, SLF= Superior longitudinal fasciculus, SCP= Superior cerebellar peduncle, ALIC= Anterior limb of internal capsule, PLIC= Posterior limb of internal capsule, ACR= Anterior corona radiata, SCR= Superior corona radiata, PCR= Posterior corona radiata, CgH= Cingulum hippocampus, CgC= Cingulum cingulate gyrus, SCC= splenium corpus callosum, FA= fractional anisotropy, MD= mean diffusivity, RD= radial diffusivity

References

1. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Semin Arthritis Rheum* 2014; 44:68-75.
2. Kim DJ, Lim M, Kim JS, Son KM, Kim HA, Chung CK. Altered white matter integrity in the corpus callosum in fibromyalgia patients identified by tract-based spatial statistical analysis. *Arthritis Rheumatol* 2014; 66:3190-3199.
3. Lieberman G, Shpaner M, Watts R, Andrews T, Filippi CG, Davis M, Naylor MR. White Matter Involvement in Chronic Musculoskeletal Pain. *Journal of Pain* 2014; 15:1110-1119.
4. Lutz J, Jager L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis Rheum* 2008; 58:3960-3969.
5. Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B. Structural and functional brain abnormalities in chronic low back pain: A systematic review. *Semin Arthritis Rheum* 2015.
6. Mansour AR, Baliki MN, Huang L, Torbey S, Herrmann KM, Schnitzer TJ, Apkarian AV. Brain white matter structural properties predict transition to chronic pain. *Pain* 2013; 154:2160-2168.
7. Buckalew N, Haut MW, Aizenstein H, Morrow L, Perera S, Kuwabara H, Weiner DK. Differences in brain structure and function in older adults with self-reported disabling and nondisabling chronic low back pain. *Pain Med* 2010; 11:1183-1197.
8. Killgore WD, Singh P, Kipman M, Pisner D, Fridman A, Weber M. Gray matter volume and executive functioning correlate with time since injury following mild traumatic brain injury. *Neurosci Lett* 2016; 612:238-244.
9. Govindarajan KA, Narayana PA, Hasan KM, Wilde EA, Levin HS, Hunter JV, Miller ER, Patel VK, Robertson CS, McCarthy JJ. Cortical Thickness in Mild Traumatic Brain Injury. *J Neurotrauma* 2016.
10. Wang X, Xie H, Cotton AS, Tamburrino MB, Brickman KR, Lewis TJ, McLean SA, Liberzon I. Early cortical thickness change after mild traumatic brain injury following motor vehicle collision. *J Neurotrauma* 2015; 32:455-463.
11. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. *Brain* 2007; 130:2508-2519.
12. Koerte IK, Hufschmidt J, Muehlmann M, Lin AP, Shenton ME. Frontiers in Neuroscience Advanced Neuroimaging of Mild Traumatic Brain Injury, in Laskowitz D, Grant G (eds). Translational Research in Traumatic Brain Injury. CRC Press/Taylor and Francis Group (c) 2016 by Taylor & Francis Group, LLC., Boca Raton (FL), 2016.
13. Davis CG. Mechanisms of chronic pain from whiplash injury. *J Forensic Leg Med* 2013; 20:74-85.
14. Alexander MP. In the pursuit of proof of brain damage after whiplash injury. *Neurology* 1998; 51:336-340.
15. Yunus MB. Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. *Semin Arthritis Rheum* 2007; 36:339-356.
16. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. *Prog Neurobiol* 2011; 93:385-404.
17. Bay E, Kalpakjian C, Giordani B. Determinants of subjective memory complaints in community-dwelling adults with mild-to-moderate traumatic brain injury. *Brain Inj* 2012; 26:941-949.
18. Nampiaparampil DE. Prevalence of chronic pain after traumatic brain injury: a systematic review. *Jama* 2008; 300:711-719.
19. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain* 2011; 152:S49-64.
20. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. *Journal of Neuroimmune Pharmacology* 2013; 8:518-534.
21. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013; 17:299-312.
22. Malfliet A, Kregel J, Cagnie B, Kuipers M, Dolphens M, Roussel N, Meeus M, Danneels L, Bramer WM, Nijs J. Lack of evidence for central sensitization in idiopathic, non-traumatic neck pain: a systematic review. *Pain Physician* 2015; 18:223-236.
23. Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. *Clin J Pain* 2005; 21:175-181.
24. Coppieters I, De Pauw R, Kregel J, Malfliet A, Goubert D, Lenoir D, Cagnie B, Meeus M. Differences Between Women With Traumatic and Idiopathic Chronic Neck Pain and Women Without Neck Pain: Interrelationships Among Disability, Cognitive Deficits, and Central Sensitization. *Phys Ther* 2017; 97:338-353.
25. De Pauw R, Coppieters I, Meeus M, Caeyenberghs K, Danneels L, B C. Is traumatic and non-traumatic neck pain associated with brain alterations? A systematic review. *Accepted in Pain Physician* 2017.
26. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J* 1994; 66:259-267.
27. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B* 1996; 111:209-219.
28. Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, Chabriat H. Diffusion tensor imaging: concepts and applications. *J Magn Reson Imaging* 2001; 13:534-546.
29. Tournier JD, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magn Reson Med* 2011; 65:1532-1556.
30. Jones DK, Leemans A. Diffusion tensor imaging. *Methods Mol Biol* 2011; 711:127-144.
31. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed* 2002; 15:435-455.
32. Suleyman Sener PPM, Maas Andrew I.R. Diffusion tensor imaging in traumatic brain injury, in Van Hecke Wim EL, Sunaert S (ed). Diffusion tensor imaging: a practical handbook. Springer, 2016, pp 373-380.
33. Rakic P. Defects of neuronal migration and the pathogenesis of cortical malformations. *Prog Brain Res* 1988; 73:15-37.
34. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician* 2015; 18:E389-401.
35. Coppieters I, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. *J Pain* 2016.
36. Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain* 2008; 131:3222-3231.
37. Ruigrok AN, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev* 2014; 39:34-50.
38. Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. *J Pain Res* 2015; 8:311-320.
39. van Hemmen J, Saris IM, Cohen-Kettenis PT, Veltman DJ, Pouwels PJ, Bakker J. Sex Differences in White Matter Microstructure in the Human Brain Predominantly Reflect Differences in Sex Hormone Exposure. *Cereb Cortex* 2016.
40. Perelle IB, Ehrman L. An international study of human handedness: the data. *Behav Genet* 1994; 24:217-227.
41. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 2006; 10:287-333.
42. Vernon H. The Neck Disability Index: state-of-the-art, 1991-2008. *J Manipulative Physiol Ther* 2008; 31:491-502.
43. Sterling M. A proposed new classification system for whiplash associated disorders--implications for assessment and management. *Man Ther* 2004; 9:60-70.
44. Jorritsma W, de Vries GE, Geertzen JH, Dijkstra PU, Reneman MF. Neck Pain and Disability Scale and the Neck Disability Index: reproducibility of the Dutch Language Versions. *Eur Spine J* 2010; 19:1695-1701.
45. Jorritsma W, de Vries GE, Dijkstra PU, Geertzen JH, Reneman MF. Neck Pain and Disability Scale and Neck Disability Index: validity of Dutch language versions. *Eur Spine J* 2012; 21:93-100.
46. Sanchez-Cubillo I, Perianez JA, Adrover-Roig D, Rodriguez-Sanchez JM, Rios-Lago M, Tirapu J, Barcelo F. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *J Int Neuropsychol Soc* 2009; 15:438-450.
47. Tombaugh TN. Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol* 2004; 19:203-214.
48. Misdraji EL, Gass CS. The Trail Making Test and its neurobehavioral components. *J Clin Exp Neuropsychol* 2010; 32:159-163.

49. Bowie CR, Harvey PD. Administration and interpretation of the Trail Making Test. *Nat Protoc* 2006; 1:2277-2281.
50. Whiteside A, Hansen S, Chaudhuri A. Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 2004; 109:497-499.
51. Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. *Scand J Rehabil Med* 1999; 31:89-93.
52. Cathcart S, Pritchard D. Reliability of pain threshold measurement in young adults. *J Headache Pain* 2006; 7:21-26.
53. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol* 2010; 23:611-615.
54. Ng TS, Pedler A, Vicenzino B, Sterling M. Less efficacious conditioned pain modulation and sensory hypersensitivity in chronic whiplash-associated disorders in Singapore. *Clin J Pain* 2014; 30:436-442.
55. Tousignant-Laflamme Y, Page S, Goffaux P, Marchand S. An experimental model to measure excitatory and inhibitory pain mechanisms in humans. *Brain Res* 2008; 1230:73-79.
56. Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. *Pain Res Manag* 2012; 17:98-102.
57. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice AS. Reliability of conditioned pain modulation: a systematic review. *Pain* 2016; 157:2410-2419.
58. Fischl B. FreeSurfer. *Neuroimage* 2012; 62:774-781.
59. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; 33:341-355.
60. Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 2004; 22:1060-1075.
61. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998; 17:87-97.
62. Fischl B, Liu A, Dale AM. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging* 2001; 20:70-80.
63. Segonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging* 2007; 26:518-529.
64. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 1999; 9:179-194.
65. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 2000; 97:11050-11055.
66. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006; 31:968-980.
67. Cauda F, Palermo S, Costa T, Torta R, Duca S, Vercelli U, Geminiani G, Torta DM. Gray matter alterations in chronic pain: A network-oriented meta-analytic approach. *Neuroimage Clin* 2014; 4:676-686.
68. Piche M, Chen JI, Roy M, Poitras P, Bouin M, Rainville P. Thicker posterior insula is associated with disease duration in women with irritable bowel syndrome (IBS) whereas thicker orbitofrontal cortex predicts reduced pain inhibition in both IBS patients and controls. *J Pain* 2013; 14:1217-1226.
69. Schwedt TJ, Chong CD. Correlations between brain cortical thickness and cutaneous pain thresholds are atypical in adults with migraine. *PLoS One* 2014; 9:e99791.
70. Erpelding N, Moayedi M, Davis KD. Cortical thickness correlates of pain and temperature sensitivity. *Pain* 2012; 153:1602-1609.
71. Leemans A JB, Sijbers J, Jones, DK. . ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data. Paper presented at: 17th Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM); Hawaii, HI.April 2009. p. pp 3537.
72. Leemans A, Jones DK. The B-matrix must be rotated when correcting for subject motion in DTI data. *Magn Reson Med* 2009; 61:1336-1349.
73. Mori S, Oishi K, Jiang H, Jiang L, Li X, Akhter K, Hua K, Faria AV, Mahmood A, Woods R, Toga AW, Pike GB, Neto PR, Evans A, Zhang J, Huang H, Miller MI, van Zijl P, Mazziotta J. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage* 2008; 40:570-582.
74. Chong CD, Plasencia JD, Frakes DH, Schwedt TJ. Structural alterations of the brainstem in migraine. *Neuroimage Clin* 2017; 13:223-227.
75. Drijkoningen D, Caeyenberghs K, Leunissen I, Vander Linden C, Leemans A, Sunaert S, Duysens J, Swinnen SP. Training-induced improvements in postural control are accompanied by alterations in cerebellar white matter in brain injured patients. *Neuroimage Clin* 2015; 7:240-251.
76. Ly M, Adluru N, Destiche DJ, Lu SY, Oh JM, Hoscheidt SM, Alexander AL, Okonkwo OC, Rowley HA, Sager MA, Johnson SC, Bendlin BB. Fornix Microstructure and Memory Performance Is Associated with Altered Neural Connectivity during Episodic Recognition. *J Int Neuropsychol Soc* 2016; 22:191-204.
77. Baek SO, Kim OL, Kim SH, Kim MS, Son SM, Cho YW, Byun WM, Jang SH. Relation between cingulum injury and cognition in chronic patients with traumatic brain injury; diffusion tensor tractography study. *NeuroRehabilitation* 2013; 33:465-471.
78. Koenig KA, Sakaie KE, Lowe MJ, Lin J, Stone L, Bermel RA, Beall EB, Rao SM, Trapp BD, Phillips MD. The relationship between cognitive function and high-resolution diffusion tensor MRI of the cingulum bundle in multiple sclerosis. *Mult Scler* 2015; 21:1794-1801.
79. Lin L, Xue Y, Duan Q, Sun B, Lin H, Chen X, Luo L, Wei X, Zhang Z. Microstructural White Matter Abnormalities and Cognitive Dysfunction in Subcortical Ischemic Vascular Disease: an Atlas-Based Diffusion Tensor Analysis Study. *J Mol Neurosci* 2015; 56:363-370.
80. DeSouza DD, Hodaie M, Davis KD. Abnormal trigeminal nerve microstructure and brain white matter in idiopathic trigeminal neuralgia. *Pain* 2014; 155:37-44.
81. Chen JY, Blankstein U, Diamant NE, Davis KD. White matter abnormalities in irritable bowel syndrome and relation to individual factors. *Brain Res* 2011; 1392:121-131.
82. Upadhyay J, Maleki N, Potter J, Elman I, Rudrauf D, Knudsen J, Wallin D, Pendse G, McDonald L, Griffin M, Anderson J, Nutile L, Renshaw P, Weiss R, Becerra L, Borsook D. Alterations in brain structure and functional connectivity in prescription opioid-dependent patients. *Brain* 2010; 133:2098-2114.
83. Borsook D, Linnman C, Faria V, Strassman AM, Becerra L, Elman I. Reward deficiency and anti-reward in pain chronification. *Neurosci Biobehav Rev* 2016; 68:282-297.
84. McAllister TW. Neuropsychiatric sequelae of head injuries. *Psychiatr Clin North Am* 1992; 15:395-413.
85. Sarikcioglu L, Ozsoy U, Unver G. Tapetum corporis callosi: carpet of the brain. *J Hist Neurosci* 2007; 16:432-434.
86. Todd E. Lempert EHLG. Pediatric neuroimaging, in Clyde A. Helms WEB (ed). Fundamentals of diagnostic radiology Lippincott williams and wilkins and wolters kluwer business, Philadelphia, USA, 2007, pp 217.
87. Meeus M, Van Oosterwijck J, Ickmans K, Baert I, Coppieters I, Roussel N, Struyf F, Pattyn N, Nijs J. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clin Rheumatol* 2015; 34:545-553.
88. Daenen L, Nijs J, Roussel N, Wouters K, Van Loo M, Cras P. Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clin Rheumatol* 2013; 32:23-31.
89. Van Oosterwijck J, Nijs J, Meeus M, Van Loo M, Paul L. Lack of endogenous pain inhibition during exercise in people with chronic whiplash associated disorders: an experimental study. *J Pain* 2012; 13:242-254.
90. Chua NHL, Timmerman H, Vissers KC, Wilder-Smith OH. Multi-modal Quantitative Sensory Testing in Patients with Unilateral Chronic Neck Pain: An Exploratory Study. *Journal of Musculoskeletal Pain* 2012; 20:292-299.
91. Zhang S, Li CS. Functional connectivity mapping of the human precuneus by resting state fMRI. *Neuroimage* 2012; 59:3548-3562.
92. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 2006; 129:564-583.
93. Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O. Mapping the structural core of human cerebral cortex. *PLoS Biol* 2008; 6:e159.
94. Fransson P, Marrelec G. The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *Neuroimage* 2008; 42:1178-1184.

95. Raichle ME. The brain's default mode network. *Annu Rev Neurosci* 2015; 38:433-447.
96. Aerts H, Fias W, Caeyenberghs K, Marinazzo D. Brain networks under attack: robustness properties and the impact of lesions. *Brain* 2016; 139:3063-3083.
97. Ichesco E, Puiu T, Hampson JP, Kairys AE, Clauw DJ, Harte SE, Peltier SJ, Harris RE, Schmidt-Wilcke T. Altered fMRI resting-state connectivity in individuals with fibromyalgia on acute pain stimulation. *Eur J Pain* 2016; 20:1079-1089.
98. Xiong KL, Zhang JN, Zhang YL, Zhang Y, Chen H, Qiu MG. Brain functional connectivity and cognition in mild traumatic brain injury. *Neuroradiology* 2016; 58:733-739.
99. Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional reorganization of the default mode network across chronic pain conditions. *PLoS One* 2014; 9:e106133.
100. Kucyi A, Salomons TV, Davis KD. Mind wandering away from pain dynamically engages antinociceptive and default mode brain networks. *Proc Natl Acad Sci U S A* 2013; 110:18692-18697.
101. Jensen KB, Srinivasan P, Spaeth R, Tan Y, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Vitton O, Gracely R, Ingvar M, Kong J. Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis Rheum* 2013; 65:3293-3303.
102. Xiong K, Zhu Y, Zhang Y, Yin Z, Zhang J, Qiu M, Zhang W. White matter integrity and cognition in mild traumatic brain injury following motor vehicle accident. *Brain Res* 2014; 1591:86-92.
103. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron* 2008; 60:570-581.
104. Veeramuthu V, Narayanan V, Kuo TL, Delano-Wood L, Chinna K, Bondi MW, Waran V, Ganesan D, Ramli N. Diffusion Tensor Imaging Parameters in Mild Traumatic Brain Injury and Its Correlation with Early Neuropsychological Impairment: A Longitudinal Study. *J Neurotrauma* 2015; 32:1497-1509.
105. K M. Curran LE, A. Leemans. Quantitative DTI measures, in Wim van Hecke LE, Stefan Sunaert (ed). Diffusion tensor imaging: a practical handbook. Springer, New York, 2016, pp 65-85.
106. Horsfield MA, Jones DK. Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review. *NMR Biomed* 2002; 15:570-577.
107. Jones DK, Christiansen KF, Chapman RJ, Aggleton JP. Distinct subdivisions of the cingulum bundle revealed by diffusion MRI fibre tracking: implications for neuropsychological investigations. *Neuropsychologia* 2013; 51:67-78.
108. Mufson EJ, Pandya DN. Some observations on the course and composition of the cingulum bundle in the rhesus monkey. *J Comp Neurol* 1984; 225:31-43.
109. Vogt BA, Pandya DN, Rosene DL. Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. *J Comp Neurol* 1987; 262:256-270.
110. Morris R, Pandya DN, Petrides M. Fiber system linking the mid-dorsolateral frontal cortex with the retrosplenial/presubicular region in the rhesus monkey. *J Comp Neurol* 1999; 407:183-192.
111. Janve VA, Zu Z, Yao SY, Li K, Zhang FL, Wilson KJ, Ou X, Does MD, Subramaniam S, Gochberg DF. The radial diffusivity and magnetization transfer pool size ratio are sensitive markers for demyelination in a rat model of type III multiple sclerosis (MS) lesions. *Neuroimage* 2013; 74:298-305.
112. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage* 2002; 17:1429-1436.
113. Sun SW, Liang HF, Schmidt RE, Cross AH, Song SK. Selective vulnerability of cerebral white matter in a murine model of multiple sclerosis detected using diffusion tensor imaging. *Neurobiol Dis* 2007; 28:30-38.
114. Wu TC, Wilde EA, Bigler ED, Yallampalli R, McCauley SR, Troyanskaya M, Chu Z, Li X, Hanten G, Hunter JV, Levin HS. Evaluating the relationship between memory functioning and cingulum bundles in acute mild traumatic brain injury using diffusion tensor imaging. *J Neurotrauma* 2010; 27:303-307.
115. Rutgers DR, Toulgoat F, Cazejust J, Fillard P, Lasjaunias P, Ducreux D. White matter abnormalities in mild traumatic brain injury: a diffusion tensor imaging study. *AJNR Am J Neuroradiol* 2008; 29:514-519.
116. Liu G, Ghimire P, Pang H, Wu G, Shi H. Improved sensitivity of 3.0 Tesla susceptibility-weighted imaging in detecting traumatic bleeds and its use in predicting outcomes in patients with mild traumatic brain injury. *Acta Radiol* 2015; 56:1256-1263.
117. Lee DH, Lee KJ, Cho KI, Noh EC, Jang JH, Kim YC, Kang DH. Brain alterations and neurocognitive dysfunction in patients with complex regional pain syndrome. *J Pain* 2015; 16:580-586.
118. Urban KJ, Riggs L, Wells GD, Keightley M, Chen JK, Ptito A, Fait P, Taha T, Sinopoli KJ. Cortical Thickness Changes and Their Relationship to Dual-Task Performance following Mild Traumatic Brain Injury in Youth. *J Neurotrauma* 2016.
119. Wheeler-Kingshott CA, Cercignani M. About "axial" and "radial" diffusivities. *Magn Reson Med* 2009; 61:1255-1260.

PART III

The relationship between cognitive performance, cognitive stress, and central sensitization in patients with chronic whiplash associated disorders and fibromyalgia.

Original research papers

Chapter 6

Cognitive performance is related to central sensitization and health-related quality of life in patients with chronic whiplash-associated disorders and fibromyalgia

Coppieters I. MSc[1,2], Ickmans K. PhD[2,3], Cagnie B. PhD[1], Nijs J. PhD[2,3,4], De Pauw R. MSc[1], Noten S. MSc[2,5], Meeus M. PhD[1,2,5]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium;
[2] Pain in Motion international research group;
[3] Departments of Human Physiology and Rehabilitation Sciences, Faculty of Physical Education & Physiotherapy, Free University of Brussels, Belgium;
[4] Department of Physical Medicine and Physiotherapy, University Hospital Brussels, Belgium; and
[5] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium.

Pain Physician, 2015; 18: 389-401.
SCI_{2015}= 3.40, 5-year impact factor= 3.694, Q2 in anesthesiology (8/31), Q2 in clinical neurology (53/193)

Abstract

Background: A growing body of research has demonstrated that impaired central pain modulation or central sensitization (CS) is a crucial mechanism for the development of persistent pain in chronic whiplash-associated disorders (WAD) and fibromyalgia (FM) patients. Furthermore, there is increasing evidence for cognitive dysfunctions among these patients. In addition, chronic WAD and FM patients often report problems with health-related quality of life (QoL). Yet, there is limited research concerning the interrelations between cognitive performance, indices of CS and health-related QoL in these patients.
Objectives: (1) Examining the presence of cognitive impairment, CS, and limitations on health-related QoL in patients with chronic WAD and FM compared to healthy controls. (2) Examining interrelations between performance-based cognitive functioning, CS, and self-reported health-related QoL in these 3 study groups.
Study design: A case-control study was conducted.
Setting: The present study took place at the University Hospital Brussels, the University of Brussels, and the University of Antwerp.
Methods: Fifty-nine participants (16 chronic WAD patients, 21 FM patients and 22 pain-free volunteers) filled out the Short Form 36 item Health Survey (SF-36), a self-reported psychosocial questionnaire, to assess health-related QoL. Next, they were subjected to various pain measurements (pressure hyperalgesia, deep-tissue hyperalgesia, temporal summation (TS), and conditioned pain modulation (CPM)). Finally, participants completed a battery of performance-based cognitive tests (Stroop task, psychomotor vigilance task (PVT), and operation span task (OSPAN)).
Results: Significant cognitive impairment, bottom-up sensitization, and decreased health-related QoL were demonstrated in patients with chronic WAD and FM compared to healthy controls (p<0.017). CPM was comparable between the 3 groups. Cognitive performance was significantly related to central pain modulation (deep-tissue hyperalgesia, TS, CPM) as well as to self-reported health-related QoL (p<0.05). Decreased cognitive performance was related to deficient central pain modulation in healthy controls. Further, significant correlations between decreased cognitive performance and reduced health-related QoL were revealed among all study groups. Additionally, FM patients showed correlations between cognitive impairment and increased health-related QoL. Remarkably, impaired selective attention and working memory were related to less TS, whereas impaired sustained attention was correlated with dysfunctional CPM in FM patients.
Limitations: Based on the current cross-sectional study no firm conclusions can be drawn on the causality of the relations.
Conclusion: In conclusion, this paper has demonstrated significant cognitive deficits, signs of CS, and reduced health-related QoL in chronic WAD and FM patients compared to healthy individuals. Significant relations between cognitive performance and CS as well as health-related QoL were demonstrated. These results provide preliminary evidence for the clinical importance of objectively measured cognitive deficits in patients with chronic WAD and FM.

Key words: Chronic pain, fibromyalgia, whiplash, central sensitization, conditioned pain modulation, temporal summation, cognition, quality of life

1. Introduction

A whiplash injury is caused by a sudden acceleration-deceleration of the head, mostly due to motor vehicle collisions [1, 2]. Up to 50% of whiplash patients develop chronic neck pain and disability [3, 4]. The term chronic whiplash-associated disorders (WAD) is used to describe the various symptoms that are experienced by whiplash patients beyond 3 months after the accident [1]. These symptoms include persistent neck pain, referred pain, headache, dizziness, emotional and cognitive disturbance, and physical dysfunctions [5-7]. Fibromyalgia (FM) is another condition characterized by various persistent symptoms [8].

The diagnosis of FM is based upon the 1990 or 2010 American College of Rheumatology (ACR) criteria [9]. According to these criteria, FM patients are characterized by chronic widespread musculoskeletal pain. Additionally, FM patients experience a variety of symptoms, including sleep disturbances, fatigue, cognitive dysfunctions, and limitations in activities of daily living [8, 10]. Chronic pain is a predominant and common debilitating symptom in both patients with WAD and FM [11-13].

Nowadays, there is compelling evidence for impaired central pain modulation or central sensitization (CS) in both patients with chronic WAD and FM as the underlying mechanism of their pain complaints [11, 13-17]. CS is defined as an exaggerated responsiveness of the central nervous system to a variety of stimuli, like pressure, temperature, light, and medication among others [18, 19]. The CS mechanism causes hyperalgesia, allodynia, temporal summation (TS), and referred pain across multiple spinal segments, leading to chronic widespread pain [11]. The augmented excitability results in a largely decreased load tolerance of the neuromusculoskeletal system. Contiguously, it has been shown that alterations in descending pain pathways are involved in the CS process [20]. Malfunctioning of descending neuronal pathways can lead to more facilitation and less inhibition of the transmitted nociceptive signals to the brain.

The conditioned pain modulation (CPM) paradigm is often used to evaluate the efficacy of endogenous pain inhibition, and relies on the "pain-inhibits-pain" mechanism [21]. Earlier studies provided evidence for inefficient CPM activation in patients with chronic WAD and FM [13, 22-24]. In addition, TS, defined as the increase in pain ratings after repetitive stimulation at a constant intensity, is widely used in pain research to assess hyperexcitability of the central nervous system [21, 25]. Interestingly, it seems that activation of CPM is able to reduce TS among healthy pain-free individuals [26]. By measuring both CPM, combined

with the evaluation of enhanced TS, important information regarding central nervous systems' pain modulation can be obtained.

Apart from persistent pain, chronic WAD and FM patients often experience cognitive deficits, including concentration difficulties and working memory deficits [1, 9, 10, 27]. More specifically, the cognitive deficits encompass longer reaction times, short-term memory deficits and attention problems [28-30]. Decreased cognitive function seems to be related to pain severity in various chronic pain populations [29, 31], and is presumed to be a feature of CS [19]. Accordingly, it is hypothesized that malfunctioning of endogenous pain inhibition and subsequent chronic pain precludes optimal cognitive performance. This hypothesis is supported by the findings of altered brain morphology [32, 33] and brain activity [34, 35] in patients with chronic WAD and FM.

Besides the growing evidence for the above mentioned dysfunctions, studies examining the relation between objectively measured cognitive performance, CS, and health-related quality of life (QoL) in patients with chronic WAD and FM are limited [28]. Accordingly, it is necessary to further investigate the possible relations between cognitive performance, CS, and health-related QoL in patients with chronic CS pain, like those with chronic WAD or FM. It is hypothesized that cognitive impairment is related to CS and increased limitations on health-related QoL.

Therefore, the aims of the current study are: 1) to compare these aspects between 2 patients groups characterized by CS, chronic WAD and FM, and healthy controls; and 2) to investigate the interrelations between cognitive performance, CS, and self-reported health-related QoL in patients with chronic WAD and FM, and healthy pain-free controls.

2. Methods

2.1. Study design and setting

The present case-control study took place at the University Hospital Brussels, the University of Brussels, and the University of Antwerp. Participants received detailed study information and gave written informed consent prior to study enrollment. All patients and healthy control subjects were unpaid volunteers. This research was approved by the Ethics committee of the University Hospital Brussels.

2.2. Participants and assessments

The present study took place from July 2010 until December 2013. Sixteen patients with chronic WAD, 21 patients with FM, and 22 healthy pain-free controls were included. Chronic WAD and FM patients were recruited in cooperation with rheumatologists and physical medicine physicians. Eligible patients, men and women, were contacted by phone and/or email. In addition, patients were contacted using social network and Internet sites of chronic WAD and FM associations. Healthy controls were recruited through friends, relatives, or acquaintances of students, researchers, patients, and university staff. Each study participant had to be Dutch speaking and aged between 18 and 65 years.
The chronic WAD group fulfilled the criteria of the Quebec Task Force (grade II to III) [1]. Chronic neck pain due to a whiplash event was defined as pain lasting longer than 3 months. The FM group complied with the diagnostic criteria for FM as defined by the 1990 ACR [9]. FM patients reporting a history of a whiplash trauma and chronic WAD patients fulfilling the diagnostic criteria for FM were excluded from the study. At the time of study participation, healthy individuals were not allowed to suffer from any pain complaints or any (chronic) disease.

General exclusion criteria were neurologic, metabolic, cardiovascular, or inflammatory disorders. In order to preclude confounding factors, pregnant women and women one year postnatal were excluded. Furthermore, all participants were asked to stop analgesics 48 hours prior to study participation, not to undertake physical exertion, and to refrain from consuming alcohol, caffeine, and nicotine on the day of the experiments.

2.3. Central sensitization

To investigate central pain modulation and the presence of CS, 4 critical aspects of the central pain system were assessed [36-39]. First, in order to evaluate local and widespread hyperalgesia, pressure pain thresholds (PPTs) were measured with a digital algometer (Wagner Instruments, Greenwich, CT, USA) at symptomatic and remote areas. Secondly, deep-tissue hyperalgesia was evaluated. Thirdly, TS of pressure pain was examined. Finally, a CPM paradigm was conducted to assess the efficacy of endogenous pain inhibition.

2.3.1. Pressure hyperalgesia

The PPT was measured at 2 different sites: the dorsal side of the intermediate phalanx of the right middle finger and the middle of the right trapezius belly, midway between the processus spinosus of the seventh cervical vertebra and the lateral edge of the acromion [40, 41]. On each site, 2 PPT measurements (interval 30 seconds) were performed, generating a mean PPT value per site. To determine the PPT, pressure was increased at a rate of approximately 1 kg/s and participants were asked to say "stop" at the moment the sensation became unpleasant. Consequently, the pressure was immediately released. The pressure established on that moment was determined as the PPT, measured in kg/cm^2. The use of pressure algometry has been found to be an efficient and reliable technique in the determination of PPTs and subsequently the examination of hyperalgesia [22, 42, 43].

2.3.2. Deep-tissue hyperalgesia

Deep-tissue hyperalgesia was investigated by inflating an occlusion cuff placed around the left arm. The cuff served also as the conditioned stimulus in the CPM paradigm (see further). Cuff inflation rate was constant (20 mmHg/s) and manually increased until the participant reported pain. The pressure at this moment was registered (cuff pressure) and

used for further data analyses. Participants then adapted to the stimulus for 30 seconds and rated the pain on the verbal numeric rating scale (VNRS). Cuff inflation was then adjusted until participants indicated pain at a level 3 of 10 on the VNRS. Subsequent, this pressure (cuff pressure VNRS3) was stored and used for further data analyses.

2.3.3. Temporal summation

TS was induced by means of a digital algometer (Wagner Instruments, Greenwich, CT, USA). TS was elicited by 10 consecutive pressure pulses at PPT intensity on the same places. For each pulse of the TS procedure, the pressure was increased at a rate of 2 kg/s until the previously determined PPT, where it was maintained for one second before being released. Pressure pulses were presented with an inter-stimulus interval of one second. Participants were instructed to rate the pain intensity of the first, fifth and tenth pressure pulse according to the VNRS. TS score was obtained by subtracting the first VNRS score from the last VNRS. The higher the TS score, the more efficient the nociceptive signaling to the brain. The TS procedure is found to be reliable and valid, and is supported to use in chronic pain patients [36].

2.3.4. Conditioned Pain Modulation

CPM was induced by inflating an occlusion cuff (conditioning stimulus) on the left arm, opposite of the test stimulus, to a painful intensity (see 2.3.2), being the TS procedure repeated while wearing the cuff.

The CPM procedure started when cuff inflation was adjusted equal to a level 3 of 10 on the VNRS. The left arm was then rested on a table while TS assessment was repeated at the right side as described above [36].

Efficacy of CPM is examined by subtracting the VNRS at the first pressure pulse prior to and during cuff inflation (CPM). The efficacy of CPM on TS was assessed by subtracting TS of pressure pain (VNRS tenth pressure pulse) prior to and during cuff inflation (CPM on TS) [28]. This CPM procedure is found to be reliable, and CPM induced by ischemic cuff inflation is able to reduce TS in healthy controls [36].

2.4. Cognitive performance

Cognitive performance was assessed using a battery of 3 consecutive computer tests: the Stroop task, the psychomotor vigilance task (PVT), and the operation span task (OSPAN). In order to standardize the procedure, each test began with the presentation of written instructions for that particular test. All study participants performed the cognitive tasks on the same computer and in a fixed order (i.e., Stroop task, PVT, and OSPAN). These cognitive tests are selected based on the outcome of a systematic review addressing cognitive performance in chronic pain patients [27]. Each of the 3 tests has been used and described in detail in 3 of our previous studies in patients with chronic CS pain [28, 44, 45].

2.4.1 The Stroop Task

The Stroop Task was used to evaluate selective attention, cognitive inhibition, and choice reaction time [46]. Three different conditions were used, namely, "incongruent" (word and ink color are different), "non word" (XXX in a specific color), and "negative priming" (e.g., the word green displayed in red immediately followed by the word blue displayed in green).

Stroop reaction times for correct responses were taken into account for further analyses. Stroop interference effect was calculated by subtracting Stroop reaction time non-word from Stroop reaction time incongruent. Stroop interference seems to reflect one's ability to inhibit irrelevant information, and is therefore a measure of cognitive inhibitory capacity.

Negative priming is defined as the condition where the to-be-ignored response in the first presentation becomes the subsequent relevant dimension. Furthermore, negative priming is believed to rely on one of the mechanisms of selective attention [47]. Hence, negative priming can provide more information about the quality of cognitive control to select relevant information.

2.4.2. The Psychomotor vigilance task

The PVT has been validated as a measure of sustained attention, alertness and simple reaction time [48]. Participants were instructed to respond as quickly as possible to a visual stimulus (red spot on a black screen) presented at a variable time-interval (2,000 - 10,000 ms). The trial was stored as a lapse, if a response had not been made within 500 ms. The PVT reaction time of correct responses and number of lapses were registered and used for statistical analyses. The PVT has good test-retest reliability for median response times (ICC=0.89, $p<0.0001$) and number of PVT lapses (ICC=0.83, $p<0.0001$) [49].

2.4.3. The Operation Span Task

The OSPAN task was used to assess working memory capacity [50]. The OSPAN task consisted of exercises on letter recall and math operation. The "Operation span" is the maximum number of letters that can be recalled. When the test was terminated, the "OSPAN total score" was retrieved and used for further statistical analyses. The "OSPAN total score" is the sum of all perfectly recalled exercise sets. This score measures working memory capacity as it indicates the number of letters recalled in the correct position.

2.5. Self-reported health-related QoL

The Short Form 36-item Health Survey (SF-36) was used to assess physical function, mental health, and health-related QoL [51]. This self-reported questionnaire examines 2 main domains of health, namely the physical and mental component. Higher scores represent better health for that particular subitem.

The SF-36 has been demonstrated to have good reliability and validity in chronic pain patients [51].

2.6. Data analysis

All statistical analyses were performed using IBM® SPSS® Statistics 22.0. Normality of variables was tested with the Shapiro-Wilk test and by visual evaluation of the histograms and QQ-plots. In addition, the Levene's test examined equality of variance. The assumption of data normality and equality of variance was not fulfilled. Accordingly, non-parametric tests were used for further data analyses. Comparability of groups for age, gender distribution, and disease duration was examined with the one-way ANOVA test and Chi-square test.

First, the median values of the SF-36 questionnaire, pain measurements, and performance-based cognitive tests were compared between the 3 study groups using the Kruskal-Wallis test. When a significance level of $p<0.05$ was found, the Mann-Whitney U test was performed for post-hoc comparisons. A significance level of $p<0.017$ ($\alpha<0.05/3$) was used (Bonferroni correction was applied to compensate for the multiple testing problem) and to maintain the initial significance level of $\alpha<0.05$.

To determine the relationship between cognitive performance, CS, and health-related QoL, Spearman correlation coefficients were calculated between the results of the cognitive tests and central pain measures and SF-36 scores, respectively.

3. Results

3.1. Group characteristics

The demographic characteristics of the 3 study groups are presented in Table 1. All study groups were comparable for age and gender distribution. Further, disease duration was not significantly different between the 2 patient groups.

3.2 Comparison between patients with chronic WAD, FM, and healthy controls

3.2.1 Central sensitization

Pressure hyperalgesia and deep-tissue hyperalgesia

Results of pressure and deep-tissue hyperalgesia are displayed in Table 2. PPTs at the shoulder and finger were significantly lower in FM patients compared to chronic WAD patients and controls. In addition, cuff pressures at the arm were significantly lower in the FM group compared to healthy participants.

Table 1 Demographic characteristics of the patients (cWAD and FM) and healthy controls.[a]

Characteristics	cWAD (n = 16)	FM (n = 21)	CON (n = 22)	*P* value ANOVA	*P* value Post-hoc Bonferroni		
					cWAD vs FM	CON vs cWAD	CON vs FM
Age (y)[b]	41.62 (11.45)	44.52 (9.47)	38.00 (13.90)	0.202	1	1	0.227
Gender (male; female)[c]	3; 13	5; 16	8; 14	0.442			
Disease duration (m)[b]	60.80 (69.70)	96.30 (73.10)	0 (0)	**< 0.001**	0.219	**0.004**[d]	**< 0.001**[e]
Occupational situation[c]	7 unemployed	16 unemployed	4 unemployed				
	1 part-time	2 part-time	4 part-time				
	8 full-time	3 full-time	9 full-time				
	0 student	0 student	5 student				
Pain medication use (yes, no)[c]	0; 16	4; 17	1; 21	0.084			
Antidepressiva use (yes, no)[c]	4; 12	7; 14	1; 21	**< 0.001**			
Other medication use (yes, no)[c]				0.448			
Benzodiazepines & muscle relaxants	1; 15	1; 20	1; 21				
Antihypertensiva	0; 16	0; 21	3; 19				
Medication for hyper- or hypothyrodism	1; 15	3; 18	0; 22				
Medication for diabetes	1; 15	1; 20	0; 22				
Anti-epileptic	0; 16	0; 21	1; 21				

a= Values are presented as means and SD for continuous data and as absolute frequencies for categorical data.
b= Statistical analyses were performed using a one-way ANOVA. c= Statistical analyses were performed using a Pearson Chi-square test. d= Significant differences between cWAD patients and controls ($p<0.017$).
e= Significant differences between FM patients and controls ($p<0.017$).
y: years, m: months, cWAD: chronic whiplash-associated disorders, FM: fibromyalgia, CON: healthy controls

Table 2 Comparison of pressure hyperalgesia, deep-tissue hyperalgesia, temporal summation, and conditioned pain modulation between patients (cWAD and FM) and healthy controls.

PAIN MEASURES	cWAD (n=16)	FM (n=21)	CON (n=22)	Kruskal-Wallis	Mann-Whitney U
	Median (IQR)	Median (IQR)	Median (IQR)	*p* value <0.05	*p* value < 0.017
Pressure and deep tissue hyperalgesia					
PPT finger	7.70 (5.40 – 9.60)	3.00 (1.89 – 5.68)	6.65 (4.47 – 9.35)	**0.002**	FM < cWAD°; FM < CON*
PPT shoulder	4.00 (2.50 – 5.20)	1.32 (1.08 – 2.60)	4.22 (2.99 – 5.80)	**< 0.001**	FM < cWAD°; FM < CON*
Cuff pressure	160.00 (90.00 – 200.00)	80.00 (63.00 – 145.00)	170 .00 (97.5 – 215)	**0.018**	FM < CON*
Cuff pressure (VNRS3)	110.00 (60.00 – 200.00)	63.00 (42 – 90)	100.00 (80 – 172)	**0.020**	FM < CON*
Temporal summation					
TS finger	3.50 (2.00 – 5.00)	2.00 (1.00 – 3.00)	2.00 (1.00 – 3.00)	**0.025**	cWAD > CON**
TS shoulder	3.00 (2.00 – 4.00)	3.00 (2.00 – 5.00)	1.00 (0.00 – 3.00)	**0.009**	FM > CON*; cWAD > CON**
Conditioned pain modulation					
CPM finger (VNRS1)	0.00 (0.00 – 2.00)	0.00 (0.00 – 1.00)	0 .00 (0.00 – 1.00)	0.620	ns
CPM shoulder (VNRS1)	1.00 (0.00 – 2.00)	0.00 (- 0.75 – 1.00)	1.00 (0.00 – 2.00)	0.080	ns
CPM on TS finger (VNRS10)	1.00 (0.00 – 3.00)	0.00 (-0.37 – 1.00)	2.00 (0.00 – 2.00)	0.070	ns
CPM on TS shoulder (VNRS10)	1.00 (0.00 – 2.50)	1.00 (0.00 – 1.75)	1.00 (0.00 – 2.00)	0.600	ns

Values are presented as median value and interquartile range (IQR). Significant differences are presented in bold font. PPT: Pressure pain threshold, VNRS: Verbal numeric rating scale, TS: Temporal summation, CPM: Conditioned pain modulation, ns= not significant, cWAD: chronic whiplash-associated disorders, FM: fibromyalgia, CON: healthy controls. Significant differences ($p<0.017$) between cWAD and FM are presented as (°) between CON and FM are presented as (*) between CON and cWAD are presented as (**).

Temporal summation and conditioned pain modulation

Results of TS prior to cuff inflation are shown in Table 2. No significant differences in TS were observed between chronic WAD and FM. In contrast, TS was significantly higher in both patient groups in comparison with healthy controls. The 3 study groups displayed no significant differences for the efficacy of endogenous pain inhibition (CPM).

3.2.2 Cognitive performance

Table 3 presents the median and interquartile ranges (IQR) of the 3 performance-based cognitive tests and their subscales for the patients and controls. FM patients presented impaired cognitive performance on all cognitive tests compared to healthy individuals. Chronic WAD patients only demonstrated impaired performance on the PVT test (significant longer PVT reaction times and more PVT lapses) compared to healthy pain-free controls.

Table 3 Comparison of cognitive performance (Stroop Task, PVT, OSPAN) between patients (cWAD and FM) and healthy controls.

COGNITIVE TESTS	cWAD (n=16)	FM (n=21)	CON (n=22)	Kruskal-Wallis	Mann-Whitney U
	Median (IQR)	Median (IQR)	Median (IQR)	*p* value < 0.05	*p* value < 0.017
Stroop reaction times (ms)					
Incongruent	1082.63 (977.52 – 1326.83)	1329.17 (1134.53 – 1605.71)	998.53 (886.49 – 1157.48)	**0.006**	FM > CON*
Non-word	1014.61 (859.03 – 1134.20)	1226.52 (1017.99 – 1384.80)	903.39 (852.98 – 1095.35)	**0.016**	FM > CON*
Stroop Interference	127.79 (29.20 – 223.60)	143.53 (65.14 – 194.91)	39.17 (6.05 – 114.59)	**0.025**	FM > CON*
Priming negative	1063.11 (954.06 – 1572.11)	1333.89 (1168.65 – 1735.66)	983.55 (884.11 – 1203.07)	**0.018**	FM > CON*
Psychomotor Vigilance Task					
Reaction time (ms)	342.92 (319.50 – 382.43)	328.20 (315.34 – 362.98)	298.05 (281.96 – 315.88)	**< 0.001**	FM > CON; cWAD > CON
Lapses	12.50 (3.50 – 25.75)	10.50 (6.00 – 15.00)	3.00 (1.50 – 4.50)	**0.001**	FM > CON; cWAD > CON
Operation Span Task					
OSPAN Total score	46.50 (15.50 – 55.50)	41.00 (28.50 – 55.00)	55.00 (49.00 – 64.00)	**0.011**	FM < CON*

Values are presented as median values and interquartile range (IQR). Significant differences are presented in bold font. cWAD: chronic whiplash-associated disorders, FM: fibromyalgia, CON: healthy controls.
Significant differences ($p<0.017$) between cWAD and FM are presented as (°) between CON and FM are presented as (*) between CON and cWAD are presented as (**).

3.2.3 Self-reported health-related QoL

Median values and interquartile ranges (IQR) of the SF-36 total score, mental and physical health summary score are presented in Table 4.

FM patients demonstrated higher limitations on all the SF-36 physical health domains compared to chronic WAD patients. In addition, both patient groups reported significantly more problems on physical and mental health compared to healthy participants.

Table 4 Comparison of self-reported health-related QoL (SF-36) between patients (cWAD and FM) and healthy controls

SF-36 QUESTIONNAIRE	cWAD (n=16) Median (IQR)	FM (n=21) Median (IQR)	CON (n=22) Median (IQR)	Kruskal-Wallis *p* value < 0.05	Mann-Whitney U *p* value < 0.017
Quality of life					
Physical health total	167.50 (134.25 – 249.50)	116.00 (76.00 – 152.50)	371.00 (336.00 – 387.75)	**< 0.001**	FM < cWAD°; FM < CON*; cWAD < CON**
Mental health total	242.25 (150.96 – 291.50)	220.50 (159.83 – 264.00)	354.50 (337.87 – 373.54)	**< 0.001**	FM < CON*; cWAD < CON **
SF-36 total score	414.25 (274.25 – 546.25)	338.00 (271.83 – 382.25)	728.00 (662.25 – 748.25)	**< 0.001**	FM < CON*; cWAD < CON **

Values are presented as median values and interquartile range (IQR). Significant differences are presented in bold. SF-36: 36-Item Short-Form Health Survey, cWAD: chronic whiplash-associated disorders, FM: fibromyalgia, CON: healthy controls. Significant differences (p<0.017) between cWAD and FM are presented as (°); between CON and FM are presented as (*); between CON and cWAD are presented as (**).

3.3 Relations between cognitive performance, CS, and health-related QoL

3.3.1. Cognitive performance and central sensitization

In the chronic WAD group, deep-tissue hyperalgesia was the only variable that significantly correlated (r= 0.517, $p< 0.05$) with cognitive performance, i.e. Stroop interference (data not shown).

FM patients showed significant relations between cognitive performance and 4 measures of CS, as presented in Table 5. Longer Stroop reaction times and decreased recall capacities on the OSPAN were significantly correlated with lower TS scores. Further, an increased number of PVT lapses was significantly correlated with lower tolerable cuff pressure (VNRS3) and less efficient endogenous pain inhibition (CPM).

In the healthy control group, longer Stroop and PVT reaction times were significantly related with respectively, less CPM efficiency and lower cuff pressure (VNRS3) as demonstrated in Table 5.

Table 5 Spearman correlations between cognitive performance and pain measures in FM patients (n=21) and healthy controls (n=22).

	StroopRT incongruent	StroopRT non-word	Stroop interference	StroopRT priming neg.	PVT RT	PVT LAPSES	OSPAN Total score
FM (n=21)							
TS finger	-.341	-.402	-.303	-.155	.213	.054	.430
TS shoulder	**-.505***	**-.529***	-.165	**-.502***	.197	-.034	**.531***
Cuff pressure	-.006	-.009	.082	-.006	-.152	-.501	.189
Cuff pressure (VNRS3)	-.094	-.141	-.050	.201	-.371	**-.625***	-.089
CPM finger (VNRS1)	-.053	-.100	.172	.142	-.214	**-.648****	-.065
CPM shoulder (VNRS1)	-.463	-.467	-.334	-.089	.150	.099	-.187
CPM on TS fing	.129	.092	.148	.393	-.240	-.427	.055
CPM on TS sh	-.298	-.307	.064	-.089	-.194	**-.530***	.075
CON (n=22)							
TS finger	-.253	-.234	-.328	-.275	-.112	-.232	.089
TS shoulder	.045	.115	-.076	.020	.280	-.029	-.162
Cuff pressure	-.189	-.279	.007	-.260	-.047	.208	.314
Cuff pressure (VNRS3)	-.292	-.323	-.136	-.379	**-.472***	-.196	.368
CPM finger (VNRS1)	-.304	-.321	-.298	-.379	-.307	.068	.292
CPM shoulder (VNRS1)	**-.579****	**-.539****	**-.448***	**-.622****	-.387	-.375	.184
CPM on TS fing	**-.483***	**-.452***	**-.495***	**-.580****	-.411	-.156	.261
CPM on TS sh	-.214	-.132	-.227	-.146	-.217	-.370	.051

Significant correlations are presented in bold. * Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed). VNRS: Verbal numeric rating scale, CPM: Conditioned pain modulation, TS: Temporal summation, RT: Reaction time, PVT: Psychomotor vigilance task, OSPAN: Operation Span Task, neg: negative, sh: shoulder, fing: finger.

Table 6 Spearman correlations between cognitive performance and health-related QoL in cWAD patients (n= 15), FM patients (n= 17), and healthy controls (n= 22).

	StroopRT incongruent	StroopRT non-word	Stroop interference	StroopRT priming neg.	PVT RT	PVT LAPSES	OSPAN Total score
cWAD							
Physical health total	-.321	-.225	-.154	-.300	-.171	.084	-.301
Mental health total	**-.543***	-.500	-.304	**-.518***	-.461	-.291	-.138
SF-36 total score	**-.529***	-.479	-.318	-.493	-.389	-.280	-.152
FM							
Physic health total	**.535***	.466	**.595***	.463	**-.585***	-.352	.038
Mental health total	.147	.059	.277	.179	-.068	-.230	-.026
SF-36 Total score	.355	.240	**.505***	.363	-.338	-.302	-.077
CON							
Physical health total	**-.576****	**-.496***	-.403	**-.513***	-.194	-.195	.172
Mental health total	**-.425***	-.403	-.357	-.357	-.165	-.359	.076
SF-36 total score	**-.563****	**-.468***	**-.472***	**-.489***	-.159	-.176	.206

Significant correlations are presented in bold. * Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

No significant relations were detected between cognitive performance and PPTs in the 3 study groups (data not shown).

3.3.2. Cognitive performance and health-related QoL

The correlations between cognitive performance and SF-36 scores are presented in Table 6. In the chronic WAD group decreased cognitive performance was significantly related with reduced health-related QoL (SF-36).

In the FM group a different pattern of correlations was seen. Stroop reaction times and interference were positively correlated with reduced QoL, whereas negative correlations were found between reduced QoL and PVT reaction times.

In the healthy control group decreased performance on the Stroop was correlated with increased limitations on health-related QoL (SF-36).

4. Discussion

The current study examined the presence of cognitive impairment, signs of CS, and health-related QoL in chronic WAD and FM patients, compared to healthy controls. Secondly, this study is the first to examine interrelations between cognitive performance, indices of CS, and self-reported health-related QoL in these 2 chronic pain populations.

Central sensitization

Remarkably, efficacy of endogenous pain inhibition (CPM) was comparable between the 3 study groups. Yet, previous studies have revealed dysfunctional CPM in patients with chronic WAD compared to healthy controls [26]. Nevertheless, significant other features of CS were demonstrated in chronic WAD and FM patients compared to controls. First, enhanced TS was shown in both patient groups. These findings of enhanced TS in chronic WAD and FM are similar to previous studies [52, 53]. Second, significant lower PPTs and decreased tolerable cuff pressures were revealed in FM patients compared to the other study groups, representing deep-tissue hyperalgesia at the arm in patients with FM but not in chronic WAD patients. In line with the results in the FM group, previous research has observed deep-tissue hyperalgesia among FM patients [48, 54]. The fact that no deep-tissue hyperalgesia was found among chronic WAD patients is in contrast with the results of Lemming et al [52], who demonstrated widespread deep-tissue hyperalgesia in patients with chronic WAD. However, the chronic WAD patients in the study of Lemming et al [52] experienced neck pain for at least 6 months and were recruited from a specific Pain and Rehabilitation Centre. Furthermore, deep-tissue hyperalgesia was measured with a computerized pneumatic cuff. Increased bottom-up sensitization, demonstrated by enhanced TS, was present in chronic WAD and FM patients compared to healthy controls. However, no significant differences were found between the 3 study groups regarding

CPM; hence the present study could not unravel impaired endogenous pain inhibition. In addition, FM patients demonstrated more pressure and deep-tissue hyperalgesia compared to chronic WAD patients.

Further, prior research has established both primary and secondary hyperalgesia, demonstrated by decreased electrical pain thresholds at the neck and lower limb in chronic WAD patients [55].

Cognitive performance

The results of the current study showed longer choice and simple reaction times in patients with FM compared to controls, as evidenced by slower response times on the Stroop and PVT, respectively. Hence, FM patients demonstrated reduced selective and sustained attention. Furthermore, we revealed longer simple reaction times in the chronic WAD group compared to controls. In addition, both patient groups showed significantly more PVT lapses in comparison with healthy individuals. This indicates that chronic WAD and FM patients tend to make more errors of omission during the PVT cognition task. In addition, longer PVT reaction times point to the failure of sustained attention.

An increased Stroop interference effect could not be demonstrated in the chronic WAD group, but the current study did however find significant increased interference and priming effect in FM patients relative to healthy controls. These results imply that chronic WAD patients are capable to inhibit irrelevant information, whereas FM patients seem to have problems with this attending ability. Increased interference effect or impaired cognitive inhibition has been demonstrated before in FM patients [56]. In addition, significantly higher negative priming effects in FM patients were observed compared to controls. Therefore, this study provides preliminary evidence that FM patients experience problems with inhibiting distraction stimuli.

Furthermore, no differences were found between chronic WAD patients and controls regarding Stroop reaction times. This indicates that chronic WAD patients have normal selective attention. As reported previously, chronic WAD patients presented only delayed information processing when there was attentional bias, i.e., when sleep-related words were shown [28]. On the contrary, the present study did demonstrate significantly longer Stroop reaction times in FM patients compared to healthy controls. The latter may indicate a general slowing down of information processing in patients with FM.

Regarding the OSPAN, chronic WAD patients showed normal working memory capacity. In contrast, FM patients established significant lower OSPAN scores compared to controls, illustrating reduced working memory capacity in FM patients. These findings are in line with accumulating evidence showing reduced working memory capacity in FM patients [57].

Self-reported health-related QoL

The current study established significant limitations on health-related QoL in patients with chronic WAD and FM compared to healthy controls. In particular, physical and mental health were impaired in these patients. Our results confirm current evidence of impaired physical and mental health in patients with chronic WAD and FM [58, 59]. Significantly worse scores in the FM group on domains of physical health were detected in comparison with chronic WAD patients. These results are in line with the literature, as FM patients score lower than other chronic pain conditions on health domains of bodily pain and vitality [58].

In summary, FM patients demonstrated more signs of CS, higher cognitive impairment, and more physical health problems compared with chronic WAD patients. Possible explanations for the latter findings are the fact that the included FM patients experienced on average 3 years longer disease symptoms compared to the chronic WAD patients. Additionally, it is reported that the medical diagnosis of FM most often implies the presence of CS [11, 60]. In contrast, chronic WAD is associated but not uniformly characterized by CS [19].

Interrelations

Table 7 depicts a clinical useful translation of the observed correlations between cognitive impairment and respectively, impaired central pain modulation and health-related QoL limitations in the 3 study groups.

Cognitive performance and central sensitization

In the chronic WAD group, deep-tissue hyperalgesia was the only variable that significantly correlated to cognitive performance, i.e., cognitive inhibition. This finding suggests that deficits in cognitive inhibition are related to less deep-tissue hyperalgesia. However, malingering, headache, intelligence, and the degree of vigilance are possible factors influencing cognitive performance in chronic WAD patients, and may explain the observed opposite relations [61]. Possible explanations for the scarcely observed relations between cognitive performance and CS in chronic WAD patients are obscure and merit further detailed research.

In contrast, FM patients showed much more significant relations between cognitive performance and various indices of CS. In summary, impairment on the Stroop and OSPAN in FM patients was unexpectedly related to lower TS values, hence less bottom-up sensitization. Possibly, these results are due to an overall decreased vigilant state in FM patients for a variety of sensory input, e.g., pressure pulses. Subsequently, the sensory and nociceptive transmission to the brain during the TS experiment could be delayed. On the other hand, in accordance with our expectations, impairment on the PVT was related to deficient CPM and increased deep-tissue hyperalgesia. Previous research has reported that working memory deficits in FM patients are related to gray matter volume changes in specific brain regions, which may indicate structural correlates of pain-cognition

interaction [33]. In addition, it seems that chronic pain in FM patients disrupts attention and induces neuroplasticity in the central nervous system [62].

In the healthy control group, less efficient pain inhibition was related to slower reaction times on the Stroop task. Furthermore, this is the first study finding a negative relation between PVT reaction times and deep-tissue hyperalgesia.

The observations in healthy controls and part of the findings in FM patients are in line with our study hypothesis of expected correlations between decreased cognitive performance and increased indices of CS.

Cognitive performance and health-related QoL

In chronic WAD patients, correlations between impaired selective attention and lower health-related QoL, in particular mental health, were demonstrated. These results are in line with our hypothesis and consistent with earlier research that observed relations between psychological functioning and cognitive performance in patients with chronic WAD [63].

A different pattern of correlations was obtained in the FM group. Remarkably, impaired selective attention and deficient cognitive inhibition were correlated with higher health-related QoL, whereas impaired sustained attention was related to increased QoL limitations.

In the healthy control group, decreased selective attention and cognitive inhibition were related to lower health-related QoL.

Table 7 Direction of the correlations between cognitive impairment and respectively, impaired central pain modulation, and self-reported health-related QoL.

	Impaired selective attention (Stroop)			Impaired sustained attention and reaction time (PVT)			Impaired working memory (OSPAN)		
	WAD	FM	CON	WAD	FM	CON	WAD	FM	CON
Deep-tissue hyperalgesia	1/~				~	~			
Increased TS		1/~						1/~	
Deficient CPM			~		~				
Reduced QoL (SF-36)	~	1/~	~	~	~	~			~

1/~: measurements are oppositely correlated, ~: measurements are correlated in the same direction, QoL: Quality of Life.
All measurements are presented in the impaired form.

Consequently, further research is warranted to investigate if CS and reduced health-related QoL lead to cognitive impairment or vice versa.

4.1. Study strengths, limitations and recommendations for further research

The strengths of the present study are the innovative aspect and the numerous observed significant correlations. Correlation coefficients which range from 0.36 to 0.67 are generally believed to represent moderate correlations (≤0.35 = weak correlation and 0.68 - 1.0 = strong correlation) [64]. In this study, all significant correlations (p<0.05) were situated between the range of 0.42 and 0.82, thus moderate to strong correlations.

When interpreting the results, the following study limitations have to be taken into account. Firstly, based on the current cross-sectional study, no firm conclusions can be drawn on the causality of the relations. In the 3 study groups, longer reaction times were correlated with lower health-related QoL. However, it is uncertain if cognitive deficits lead to impaired QoL or vice versa. Moreover, malingering, concentration, education level, and IQ could have influenced the cognitive study results. Secondly, when conducting this study various confounders, including medication use were taken into account. However, it has to be noticed that antidepressiva use was significantly different between the 3 study groups. In addition, we cannot exclude possible differences in education level or other biopsychosocial characteristics between the patients and controls and that this may have created bias in the results. Thirdly, only non-parametric statistical analyses were performed because the sample size of the current study was rather small.

5. Conclusion

In conclusion, chronic WAD and FM patients encounter significant cognitive impairment, signs of CS, and decreased health-related QoL compared to healthy pain-free individuals. The current study revealed more indices of CS, higher cognitive impairment, and more limitations on health-related QoL in FM patients compared with chronic WAD patients. In particular, FM patients showed higher impairment of self-reported physical health, pressure and deep-tissue hyperalgesia, hampered selective attention, and reduced working memory capacity in comparison with chronic WAD patients.

Significant correlations between cognitive impairment and indices of CS and self-reported health-related QoL, respectively, were demonstrated among the 3 study groups. Especially in FM patients cognitive impairment appeared to be related to indices of CS. Reduced selective and sustained attention, as well as reduced working memory were correlated with less TS, so less bottom-up sensitization in FM. However, impaired sustained attention was related to increased deep-tissue hyperalgesia, deficient CPM, and reduced QoL in FM patients.

Accordingly, these results provide preliminary evidence for the clinical importance of objectively measured cognitive deficits in patients with chronic WAD and FM and the

relation with CS in FM. Furthermore, in both patient groups there are distinct relations between self-reported health-related QoL and cognitive performance, albeit the specific cause-effect relationship remains unclear and requires further research.

References

1. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine* 1995; 20(8 Suppl):1s-73s.
2. Ivancic PC, Panjabi MM, Ito S. Cervical spine loads and intervertebral motions during whiplash. *Traffic injury prevention* 2006; 7(4):389-99.
3. Kamper SJ, Rebbeck TJ, Maher CG, McAuley JH, Sterling M. Course and prognostic factors of whiplash: a systematic review and meta-analysis. *Pain* 2008; 138(3):617-29.
4. Carroll LJ, Holm LW, Hogg-Johnson S, Cote P, Cassidy JD, Haldeman S, Nordin M, Hurwitz EL, Carragee EJ, van der Velde G, Peloso PM, Guzman J. Course and prognostic factors for neck pain in whiplash-associated disorders (WAD): results of the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated Disorders. *Spine* 2008;33(4 Suppl):S83-92.
5. Storaci R, Manelli A, Schiavone N, Mangia L, Prigione G, Sangiorgi S. Whiplash injury and oculomotor dysfunctions: clinical-posturographic correlations. *European spine journal* 2006; 15(12):1811-6.
6. Hinoki M. Vertigo due to whiplash injury: a neurotological approach. *Acta oto-laryngologica Supplementum* 1984; 419:9-29.
7. Lovell ME, Galasko CS. Whiplash disorders--a review. *Injury* 2002;33(2):97-101.
8. Clauw DJ. Fibromyalgia: a clinical review. *JAMA* 2014; 311(15):1547-55.
9. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis and rheumatism* 1990; 33(2):160-72.
10. Seo J, Kim SH, Kim YT, Song HJ, Lee JJ, Kim SH, Han SW, Nam EJ, Kim SK, Lee HJ, Lee SJ, Chang Y. Working memory impairment in fibromyalgia patients associated with altered frontoparietal memory network. *PloS one* 2012; 7(6):e37808.
11. Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clinical rheumatology* 2007; 26(4):465-73.
12. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis care & research* 2010; 62(5):600-10.
13. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *European journal of pain (London, England)* 2013;17(3):299-312.
14. Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. *The Clinical journal of pain* 2005; 21(2):175-81.
15. Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, Curatolo M. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. *Pain* 2004; 107(1-2):7-15.
16. Chien A, Sterling M. Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. *Manual therapy* 2010; 15(1):48-53.
17. Stone AM, Vicenzino B, Lim EC, Sterling M. Measures of central hyperexcitability in chronic whiplash associated disorder--a systematic review and meta-analysis. *Manual therapy* 2013; 18(2):111-7.
18. Staud R, Smitherman ML. Peripheral and central sensitization in fibromyalgia: pathogenetic role. *Current pain and headache reports* 2002;6(4):259-66.
19. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Manual therapy* 2010; 15(2):135-41.
20. Zusman M. Forebrain-mediated sensitization of central pain pathways: 'non-specific' pain and a new image for MT. *Manual therapy* 2002; 7(2):80-8.
21. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, Hansson P, Lautenbacher S, Marchand S, Wilder-Smith O. Recommendations on terminology and practice of psychophysical DNIC testing. *European journal of pain (London, England)* 2010; 14(4):339.
22. Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. *Scandinavian journal of rehabilitation medicine* 1999; 31(2):89-93.

23. Lautenbacher S, Rollman GB. Possible deficiencies of pain modulation in fibromyalgia. *The Clinical journal of pain* 1997; 13(3):189-96.
24. Julien N, Goffaux P, Arsenault P, Marchand S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* 2005; 114(1-2):295-302.
25. Bouhassira D, Gall O, Chitour D, Le Bars D. Dorsal horn convergent neurones: negative feedback triggered by spatial summation of nociceptive afferents. *Pain* 1995; 62(2):195-200.
26. Daenen L, Nijs J, Roussel N, Wouters K, Van Loo M, Cras P. Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clinical rheumatology* 2013; 32(1):23-31.
27. Nederhof E, Lemmink KA, Visscher C, Meeusen R, Mulder T. Psychomotor speed: possibly a new marker for overtraining syndrome. *Sports medicine (Auckland, NZ)* 2006; 36(10):817-28.
28. Meeus M, Van Oosterwijck J, Ickmans K, Baert I, Coppieters I, Roussel N, Struyf F, Pattyn N, Nijs J. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clinical rheumatology* 2015; 34:545-553.
29. Antepohl W, Kiviloog L, Andersson J, Gerdle B. Cognitive impairment in patients with chronic whiplash-associated disorder--a matched control study. *NeuroRehabilitation* 2003; 18(4):307-15.
30. Tesio V, Torta DM, Colonna F, Leombruni P, Ghiggia A, Fusaro E, Geminiani GC, Torta R, Castelli L. Are Fibromyalgia patients cognitively impaired? Objective and subjective neuropsychological evidence. *Arthritis care & research* 2014; (EPub ahead of Print).
31. Weiner DK, Rudy TE, Morrow L, Slaboda J, Lieber S. The relationship between pain, neuropsychological performance, and physical function in community-dwelling older adults with chronic low back pain. *Pain medicine* (Malden, Mass) 2006; 7(1):60-70.
32. Schmidt-Wilcke T, Luerding R, Weigand T, Jurgens T, Schuierer G, Leinisch E, Bogdahn U. Striatal grey matter increase in patients suffering from fibromyalgia--a voxel-based morphometry study. *Pain* 2007; 132 Suppl 1:S109-16.
33. Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain* 2008; 131(Pt 12):3222-31.
34. Linnman C, Appel L, Soderlund A, Frans O, Engler H, Furmark T, Gordh T, Långström B, Fredrikson M. Chronic whiplash symptoms are related to altered regional cerebral blood flow in the resting state. *European journal of pain (London, England)* 2009; 13(1):65-70.
35. Cifre I, Sitges C, Fraiman D, Munoz MA, Balenzuela P, Gonzalez-Roldan A, Martínez-Jauand M, Birbaumer N, Chialvo DR, Montoya P. Disrupted functional connectivity of the pain network in fibromyalgia. *Psychosomatic medicine* 2012; 74(1):55-62.
36. Cathcart S, Winefield AH, Rolan P, Lushington K. Reliability of temporal summation and diffuse noxious inhibitory control. *Pain research & management* 2009; 14(6):433-8.
37. Staud R, Robinson ME, Price DD. Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. *The journal of pain* 2007; 8(11):893-901.
38. Meeus M, Nijs J, Huybrechts S, Truijen S. Evidence for generalized hyperalgesia in chronic fatigue syndrome: a case control study. *Clinical rheumatology* 2010; 29(4):393-8.
39. Fernandez-Carnero J, Fernandez-de-Las-Penas C, de la Llave-Rincon AI, Ge HY, Arendt-Nielsen L. Widespread mechanical pain hypersensitivity as sign of central sensitization in unilateral epicondylalgia: a blinded, controlled study. *The Clinical journal of pain* 2009; 25(7):555-61.
40. Whiteside A, Hansen S, Chaudhuri A. Exercise lowers pain threshold in chronic fatigue syndrome. *Pain* 2004; 109(3):497-9.
41. Meeus M, Roussel NA, Truijen S, Nijs J. Reduced pressure pain thresholds in response to exercise in chronic fatigue syndrome but not in chronic low back pain: an experimental study. *Journal of rehabilitation medicine* 2010; 42(9):884-90.
42. Vanderweeen L, Oostendorp RA, Vaes P, Duquet W. Pressure algometry in manual therapy. *Manual therapy* 1996; 1(5):258-65.
43. Antonaci F, Sand T, Lucas GA. Pressure algometry in healthy subjects: inter-examiner variability. *Scandinavian journal of rehabilitation medicine* 1998; 30(1):3-8.

44. Ickmans K, Clarys P, Nijs J, Meeus M, Aerenhouts D, Zinzen E, Aelbrecht S, Meersdom G, Lambrecht L, Pattyn N. Association between cognitive performance, physical fitness, and physical activity level in women with chronic fatigue syndrome. *Journal of rehabilitation research and development* 2013; 50(6):795-810.
45. Ickmans K, Meeus M, Kos D, Clarys P, Meersdom G, Lambrecht L, Pattyn N, Nijs J. Cognitive performance is of clinical importance, but is unrelated to pain severity in women with chronic fatigue syndrome. *Clinical rheumatology* 2013; 32(10):1475-85.
46. MacLeod CM. Half a century of research on the Stroop effect: an integrative review. *Psychological bulletin* 1991; 109(2):163-203.
47. Tipper SP. The negative priming effect: inhibitory priming by ignored objects. *The Quarterly journal of experimental psychology* 1985; 37(4):571-90.
48. Lim J, Dinges DF. Sleep deprivation and vigilant attention. *Annals of the New York Academy of Sciences* 2008; 1129:305-22.
49. Dorrian J RN, Dinges DF. Psychomotor vigilance performance: neurocognitive assay sensitive to sleep loss. *Sleep deprivation: clinical issues, pharmacology and sleep loss effects*. New York: In: Kushida C (ed) Marcel Dekker; 2005; P. 39-70.
50. Conway AR, Engle RW. Individual differences in working memory capacity: more evidence for a general capacity theory. *Memory (Hove, England)* 1996; 4(6):577-90.
51. Ware JE SK, Kosinski M, Gandek B. SF-36 Health Survey Manual and Interpretation Guide. Boston: MA: New England Medical Center, the Health Institute; 1993.
52. Lemming D, Graven-Nielsen T, Sorensen J, Arendt-Nielsen L, Gerdle B. Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women. *Journal of rehabilitation medicine* 2012; 44(8):648-57.
53. Staud R, Bovee CE, Robinson ME, Price DD. Cutaneous C-fiber pain abnormalities of fibromyalgia patients are specifically related to temporal summation. *Pain* 2008; 139(2):315-23.
54. Jespersen A, Dreyer L, Kendall S, Graven-Nielsen T, Arendt-Nielsen L, Bliddal H, Danneskiold-Samsoe B. Computerized cuff pressure algometry: A new method to assess deep-tissue hypersensitivity in fibromyalgia. *Pain* 2007; 131(1-2):57-62.
55. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP. Central hypersensitivity in chronic pain after whiplash injury. *The Clinical journal of pain* 2001; 17(4):306-15.
56. Veldhuijzen DS, Sondaal SF, Oosterman JM. Intact cognitive inhibition in patients with fibromyalgia but evidence of declined processing speed. *The journal of pain* 2012; 13(5):507-15.
57. Dick BD, Verrier MJ, Harker KT, Rashiq S. Disruption of cognitive function in fibromyalgia syndrome. *Pain* 2008; 139(3):610-6.
58. Salaffi F, Sarzi-Puttini P, Girolimetti R, Atzeni F, Gasparini S, Grassi W. Health-related quality of life in fibromyalgia patients: a comparison with rheumatoid arthritis patients and the general population using the SF-36 health survey. *Clinical and experimental rheumatology* 2009; 27(5 Suppl 56):S67-74.
59. Wallin MK, Raak RI. Quality of life in subgroups of individuals with whiplash-associated disorders. *European journal of pain (London, England)* 2008; 12(7):842-9.
60. Nielsen LA, Henriksson KG. Pathophysiological mechanisms in chronic musculoskeletal pain (fibromyalgia): the role of central and peripheral sensitization and pain disinhibition. *Best practice & research Clinical rheumatology* 2007; 21(3):465-80.
61. Schmand B, Lindeboom J, Schagen S, Heijt R, Koene T, Hamburger HL. Cognitive complaints in patients after whiplash injury: the impact of malingering. *Journal of neurology, neurosurgery, and psychiatry* 1998; 64(3):339-43.
62. Bertolucci PH, de Oliveira FF. Cognitive impairment in fibromyalgia. *Current pain and headache reports* 2013; 17(7):344.
63. Bosma FK, Kessels RP. Cognitive impairments, psychological dysfunction, and coping styles in patients with chronic whiplash syndrome. *Neuropsychiatry, neuropsychology, and behavioral neurology* 2002; 15(1):56-65.
64. Horne JA, Pettitt AN. High incentive effects on vigilance performance during 72 hours of total sleep deprivation. *Acta psychologica* 1985; 58(2):123-39.

Chapter 7

Effects of cognitive stress and relaxation on central pain modulation in chronic whiplash and fibromyalgia patients compared to healthy controls

Coppieters I. MSc[1,2], Cagnie B. PhD[1], Nijs J. PhD[2-4], Van Oosterwijck J. PhD[1-3], Danneels L. PhD[1], De Pauw R. MSc[1], Meeus M. PhD[1,2,5]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium;
[2] Pain in Motion international research group, www.paininmotion.be;
[3] Departments of Human Physiology and Physiotherapy, Faculty of Physical Education & Physiotherapy, Free University of Brussels, Belgium;
[4] Department of Physical Medicine and Physiotherapy, University Hospital Brussels, Belgium;
[5] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium.

Pain Physician, 2016; 19: 119-130.
SCI_{2015}= 3.40, 5-year impact factor= 3.694, Q2 in anesthesiology (8/31), Q2 in clinical neurology (53/193)

Abstract

Background: Compelling evidence has demonstrated that impaired central pain modulation contributes to persistent pain in patients with chronic whiplash associated disorders (WAD) and fibromyalgia (FM). However, there is limited research concerning the influence of stress and relaxation on central pain modulation in patients with chronic WAD and FM.

Objectives: The present study aims to investigate the effects of acute cognitive stress and relaxation on central pain modulation in chronic WAD and FM patients compared to healthy individuals.

Study Design: A randomized crossover design was employed.

Setting: The present study took place at the University of Brussels, the University Hospital Brussels, and the University of Antwerp.

Methods: Fifty-nine participants (16 chronic WAD patients, 21 FM patients, 22 pain-free controls) were enrolled and subjected to various pain measurements. Temporal summation (TS) of pain and conditioned pain modulation (CPM) were evaluated. Subsequently, participants were randomly allocated to either a group that received progressive relaxation therapy or a group that performed a battery of cognitive tests (= cognitive stressor). Afterwards, all pain measurements were repeated. One week later participant groups were switched.

Results: A significant difference was found between the groups in the change in TS in response to relaxation (p=0.008) and cognitive stress (p=0.003). TS decreased in response to relaxation and cognitive stress in chronic WAD patients and controls. In contrast, TS increased after both interventions in FM patients. CPM efficacy decreased in all 3 groups in response to relaxation (p=0.002) and cognitive stress (p=0.001).

Limitations: The obtained results only apply for a single session of muscle relaxation therapy and cognitive stress, whereby no conclusions can be made for effects on pain perception and modulation of chronic cognitive stress and long-term relaxation therapies.

Conclusions: A single relaxation session as well as cognitive stress may have negative acute effects on pain modulation in patients with FM, while cognitive stress and relaxation did not worsen bottom-up sensitization in chronic WAD patients and healthy persons. However, endogenous pain inhibition, assessed using a CPM paradigm, worsened in chronic WAD and FM patients, as well as in healthy people following both interventions.

Key words: Chronic pain, central sensitization, endogenous pain inhibition, temporal summation of pain, cognitive stressor, relaxation, fibromyalgia, whiplash-associated disorders

Introduction

Nowadays, there is compelling evidence for impaired central pain modulation or central sensitization (CS) as the common underlying pathophysiological mechanism of chronic pain in conditions such as chronic whiplash associated disorders (WAD) and fibromyalgia (FM) [1-3]. CS is defined as an exaggerated responsiveness of the central nervous system to nociceptive and non-nociceptive stimuli, like pressure, electrical stimuli, temperature, light, and medication [4-8].

Enhanced bottom-up sensitization, being an exaggerated efficient nociceptive transmission, is a possible feature in CS [9,10]. To assess the efficacy of this bottom-up sensitization, evaluation of temporal summation (TS), characterized by the increase in pain ratings after repetitive noxious stimulation at a constant intensity, has frequently been performed [11,12]. In addition, it has been shown that malfunctioning of descending pain-inhibitory pathways is involved in the CS process [13]. This can lead to increased nociceptive transmission to the brain because of the lack of dampening or filtering of the incoming information. The conditioned pain modulation (CPM) paradigm is often used to evaluate the efficacy of endogenous pain inhibition, and relies on the "pain-inhibits-pain" mechanism [12]. Enhanced TS of pain [14,15], impaired endogenous pain inhibition [16-19], and inefficient CPM [20,21] have been demonstrated in patients with chronic WAD and FM.

Furthermore, a growing body of research shows abnormalities in stress-regulating systems in chronic pain patients, including WAD and FM [22-24]. It has been demonstrated that stress can have a major impact on pain perception [25,26] by either suppressing pain (stress-induced analgesia) or exacerbating it (stress-induced hyperalgesia) [25,27-29]. Stress-induced analgesia during exercise is presumed to result from the release of endogenous opioids and growth factors [30,31] and activation of nociceptive inhibitory mechanisms orchestrated by the brain [32,33]. Previous research has demonstrated dysfunctional exercise-induced analgesia in chronic WAD and FM patients [17-19].

The exact mechanisms involved in stress-induced hyperalgesia have to be further unravelled [34]. To date, it is suggested that neurotransmitters and neuroendocrine alterations play a role in this phenomenon. In addition, alterations in brain pathways mediating excitatory and inhibitory systems likely give rise to stress-induced hyperalgesia [34].

Apart from persistent pain, chronic WAD and FM patients frequently complain of cognitive disturbances, including concentration and memory problems [35-40]. Decreased cognitive function seems to be related to pain severity in patients with chronic WAD and FM [40-42], and is presumed to be a feature of CS [3,5].

Interestingly, an overlap exists in brain regions involved in cognitive function and areas of the pain matrix (e.g., periaqueductal gray, anterior cingulate cortex) [40]. However, the influence of cognitive stress on central pain modulation has not yet been clearly described in patients with CS pain.

Inversely, there is conflicting evidence regarding the effects of relaxation therapy on pain ratings [43]. There is a lack of research concerning the effects of relaxation on central pain modulation in chronic pain patients. Further it is unclear whether stress and relaxation have similar or different effects on pain modulation in these patients. An example for a non-stressful intervention is the progressive muscle relaxation therapy (PRT) [44].

Possibly, performing cognitive challenging tasks may serve as a stressor for patients already suffering concentration and memory problems, which may further burden the central nervous system leading to enhanced disinhibition and more pain. On the contrary, it is hypothesized that cognitive stress, caused by cognitive tasks, can diminish pain ratings as a result of the so-called "distraction effect", described by Eccleston and Crombez [45].

Further, it is hypothesized that muscles are more relaxed after the PRT, leading to temporary pain relief [46]. On the contrary, another hypothesis is that the PRT leads to more body awareness, leading to more pain.

The present study aimed at investigating the effects of cognitive tasks (to induce cognitive stress) and a single relaxation session on central pain modulation in patients with chronic WAD and FM compared to healthy pain-free individuals.

Methods

Study design and setting

A randomized crossover design was employed as illustrated in Fig. 1. The present experimental study took place from July 2010 until December 2013 at the University of Antwerp, the University of Brussels, and the University Hospital Brussels. Participants received detailed study information and gave written informed consent prior to study enrollment. This research was approved by the Ethics Committee of the University Hospital Brussels. The current study is registered with the ClinicalTrials.gov Identifier number NCT01172795.

Participants

Sixteen patients with chronic WAD, 21 patients with FM, and 22 healthy pain-free controls were included. Chronic WAD and FM patients were recruited in cooperation with rheumatologists and physical medicine and rehabilitation physicians. Healthy controls were recruited through acquaintances of patients, students, researchers, and university staff. Each study participant had to be Dutch speaking and aged between 18 and 65 years.
The chronic WAD group fulfilled the criteria of the Quebec Task Force (grade II to III) [35]. Chronic neck pain due to a whiplash event was defined as pain lasting longer than 3 months. The FM group complied with the diagnostic criteria for FM as defined by the 1990 American College of Rheumatology (ACR) [36,47]. Chronic WAD patients fulfilling the diagnostic criteria for FM and FM patients reporting a history of a whiplash trauma were

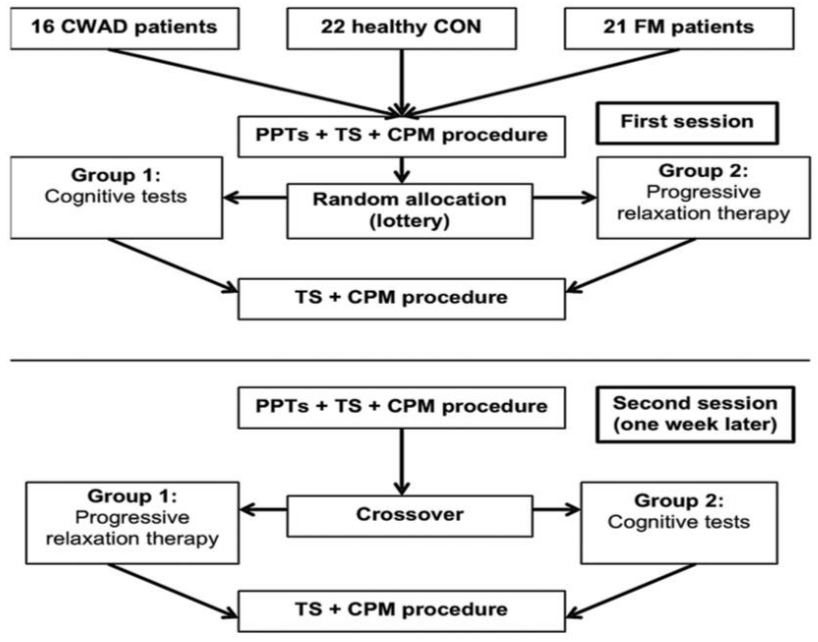


Figure 1 Flow diagram showing the study protocol.

CWAD: chronic whiplash associated disorders, CON: pain-free controls, FM: fibromyalgia, PPTs: pressure pain thresholds, TS: temporal summation, CPM: conditioned pain modulation.

not eligible for study participation. Healthy individuals were pain-free at the time of study participation. In addition, participants suffering metabolic, cardiovascular, or inflammatory disorders were excluded.

In order to preclude confounding factors, pregnant women and women less than one year postnatal were excluded. Furthermore, all participants were asked to stop analgesics 48 hours prior to study participation, not to undertake physical exertion, and to refrain from consuming caffeine, alcohol, and nicotine on the day of the experiments.

Based on an a priori power calculation, we aimed at recruiting a total sample size of at least 45 participants (G*Power 3.1.2). This a priori power analysis was performed for the within-between interaction in repeated measures ANOVA with 3 groups, 3 measurements (baseline mean pain measures, pain measures after relaxation, pain measures after cognitive tests), an effect size of 0.25, a significance level of 0.05, and a minimum power of 0.90.

Research procedure

First, participants were subjected to various pain measurements. Pressure pain thresholds (PPTs), TS, and CPM were evaluated. Subsequently, participants were randomly allocated (by lottery) to either a group that performed a battery of cognitive tests or a group that received PRT. To randomize, each participant chose a folded ticket, which indicated the order of the intervention, on the first test day. Afterwards, all pain measurements were repeated. One week later participant groups were switched.

Experimental pain measures

To investigate the presence of CS, 3 critical aspects of the central pain system were assessed [48-51]. First, PPTs were measured with a digital algometer (Wagner Instruments, Greenwich). Secondly, TS of pain was examined. Finally, a CPM paradigm was conducted to assess the efficacy of endogenous pain inhibition. All pain measurements were performed by a researcher blinded to the group allocation.

Pressure pain thresholds and temporal summation of pressure pain

The PPT was measured at the middle of the right trapezius belly, midway between the processus spinosus of the seventh cervical vertebra and the lateral edge of the acromion using a digital algometer (Wagner Instruments, Greenwich). The pressure was increased at a rate of approximately 1 kg/s until participants said "stop" at the moment the sensation became uncomfortable. Consequently, the pressure was immediately released. The pressure established on that moment was determined as the PPT, measured in kg/cm^2. Two PPT measurements (interval 30 seconds) were performed, from which a mean PPT value was calculated. The use of pressure algometry has been found to be an efficient and reliable technique in the determination of PPTs and subsequently the examination of hyperalgesia [52-54].

TS of pressure pain was elicited at the trapezius by administering 10 consecutive pressure pulses using the algometer. For each pulse of the TS procedure, the pressure was increased at a rate of approximately 2 kg/s until the previously determined PPT was reached and maintained for one second [48]. Pressure pulses were presented with an inter-stimulus interval of one second. Participants were instructed to rate their perceived pain intensity during the first, fifth and tenth pressure pulse using a verbal numeric rating scale (VNRS). The TS pain score was obtained by subtracting the first VNRS score from the last VNRS. The higher the TS score, the more extensive/efficacious the nociceptive transmission to the brain. This TS procedure has been found to be reliable and valid [48].

Conditioned pain modulation

CPM was induced by inflating an occlusion cuff (conditioning stimulus) placed around the left arm, opposite of the test stimulus, to a painful intensity. The test stimulus was applied at the contralateral body side and consisted of the TS procedure, which was repeated during cuff inflation. Therefore, the cuff was inflated at a constant rate (20 mmHg/s) until the participant reported pain. Participants then adapted to the stimulus for 30 seconds and rated the pain on the VNRS. Subsequently, the cuff inflation was adjusted until participants indicated a pain intensity of 3 out of 10 on the VNRS. The CPM procedure started as soon as the cuff inflation was adjusted. During the CPM procedure the left arm rested on a table and the TS assessment was repeated at the right trapezius as described above [48]. Efficacy of CPM was examined by subtracting the VNRS from the first pressure pulse prior to and during cuff inflation. This CPM procedure has been found reliable, and CPM induced by ischemic cuff inflation is able to reduce TS in healthy controls [48] and has been previously used to examine CPM efficacy in CWAD [55,56] and FM [57].

Interventions

Progressive muscle relaxation therapy

The relaxation intervention consisted of PRT. The participant was positioned in a comfortable supine position on a treatment table. A Dutch audio fragment was played and the participant listened to the instructions that were given. The participants were instructed to alternately contract and relax different skeletal muscle groups in order to create awareness of muscle tension and relaxation. The participant was guided to progressively proceed through all major muscle groups, relaxing them one at a time, and eventually leading to total muscle relaxation [44,58]. The relaxation session had a duration of 30 minutes.

Cognitive stress

The cognitive stress intervention encompassed the performance of 3 cognitive tests, the Stroop task, Psychomotor vigilance task (PVT), and Operation span (OSPAN) task. In order to standardize the procedure, all tests were conducted on the same computer and in a fixed order (Stroop, PVT, OSPAN). The cognitive tests were quite challenging and had a total duration of approximately 30 to 45 minutes. Each of the 3 tests has been used and described in detail in 3 of our previous studies in patients with chronic CS pain [59-61].

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences 22.0 (SPSS Inc. Headquarters, Chicago, Illinois, USA). Statistical significance was set a priori at $\alpha = 0.05$.

Comparability of groups for age, gender distribution, disease duration, and medication use was examined with the one-way ANOVA or Chi-square test. First, a paired-samples t-test was performed to ensure there were no significant differences between the baseline pain measurements at the day of the relaxation and cognitive intervention. Then, the mean of the two baseline measures (before relaxation and before cognitive intervention) of each pain measurement was calculated and used for further data analyses.

Repeated measures ANOVA were performed using 3 levels. The first level was the mean baseline pain measure, calculated as described above. The second level was the pain measure after relaxation. The third level was the same pain measure, however acquired after the cognitive tests.

First, possible interaction effects between each pain measure and "study group" were explored. If there was no significant interaction effect, the main within and between subject effects were inspected. To see the nature of the effects, a simple contrast was examined and the first level was set as the reference category. Group differences were further explored with a one-way ANOVA test. Bonferroni correction was used as post-hoc test.

Results

Baseline characteristics

Demographic characteristics, medication use, and baseline pain measures of the participants are presented in Table 1. Fifty-nine participants (16 chronic WAD patients, 21 FM patients, and 22 pain-free controls), comparable for age and gender distribution, were included for the baseline measures on the first session day and were randomly assigned. Fifty-seven participants (15 chronic WAD patients, 20 FM patients and 22 controls) were included on the second session day and were subjected to the pain measures and intervention. Two patients (1 chronic WAD, 1 FM) were lost to follow-up because they did not show up at the second intervention day. Consequently, there are 2 missing values for the TS and CPM measurements.

The paired-samples t-test in each study group displayed no significant differences between the baseline pain measurements at the experimental day of the relaxation and cognitive intervention (p>0.05) (Table 1).

Effects of relaxation and cognitive stress on central pain modulation

Temporal summation of pressure pain

A significant interaction effect was found for the change in TS between study groups (Table 2) after relaxation (p=0.008) and after the cognitive tests (p=0.003) (Table 3 and Fig. 2).

TS, measured at the trapezius muscle, decreased significantly in response to the relaxation and cognitive stress intervention in healthy persons (p<0.01). Additionally, chronic WAD patients displayed a trend for reduced TS scores after both interventions. In contrast, TS showed a trend to increase in response to the relaxation and cognitive stress intervention in the FM group.

Conditioned pain modulation

A significant main within-subjects time effect was found for CPM in every study group (Table 2), being a decreased CPM efficacy after relaxation (p=0.002) and after the cognitive tests (p=0.001) (Table 3 and Fig. 3).

Table 1 Baseline characteristics of the patients (CWAD and FM) and healthy controls[a].

Characteristics[a]		CWAD (n=16)	FM (n=21)	CON (n=22)	*p* value ANOVA	*p* value Post-hoc Bonferroni		
						CWAD - FM	CON - CWAD	CON -FM
Age (y)[b]		41.62 (11.45)	44.52 (9.47)	38.00 (13.90)	0.202			
Gender (male; female)[c]		3 ; 13	5 ; 16	8 ; 14	0.442			
Disease duration (m)[b]		60.80 (69.70)	96.30 (73.10)	0 (0)	**<0.001**	0.219	**0.004**[d]	**<0.001**[e]
Pain medication use (yes, no)[c]		0 ; 16	4 ; 17	1 ; 21	0.084			
Antidepressiva use (yes, no)[c]		4 ; 12	7 ; 14	1 ; 21	**<0.001**	0.583	0.066	**0.015**[e]
Benzodiazepines & muscle relaxants (yes, no)[c]		1 ; 15	1 ; 20	1 ; 21				
Baseline pain measure[f]								
PPT trapezius (kg/cm^2)	**relaxation**							
		4.12 (1.65)	2.90 (2.49)	5.32 (3.28)				
	cognition							
		4.02 (1.53)	2.47 (2.97)	4.96 (3.33)				
Paired samples t-test		0.741	0.264	0.384				
TS (VNRS)	**relaxation**							
		3.37 (1.94)	4.21 (2.07)	2.20 (1.86)				
	cognition							
		3.23 (1.68)	3.42 (1.80)	1.77 (1.80)				
Paired samples t-test		0.823	0.203	0.168				
CPM (VNRS)	**relaxation**							
		0.69 (1.31)	0.52 (1.50)	0.59 (1.50)				
	cognition							
		0.93 (0.88)	0.35 (1.98)	0.91 (0.97)				
Paired samples t-test		0.578	0.739	0.390				

a= Values are presented as means and SD for continuous data and as absolute frequencies for categorical data. b= Statistical analyses were performed using a one-way ANOVA. c= Statistical analyses were performed using a Pearson Chi-square test. d= Significant differences between CWAD patients and controls (p<0.017). e= Significant differences between FM patients and controls (p<0.017). f= Values are presented as mean value and SD. Significant differences are presented in bold font. y: years, m: months, PPT: Pressure pain threshold, VNRS: Verbal numeric rating scale, TS: Temporal summation, CPM: Conditioned pain modulation, ns= not significant, CWAD: chronic whiplash-associated disorders, FM: fibromyalgia, CON: healthy controls.

CPM efficacy diminished significantly after both interventions in healthy persons ($p<0.05$). Furthermore, chronic WAD patients demonstrated significantly decreased CPM efficacy after the PRT ($p<0.05$).

Table 2 Main effects: Repeated measures analysis of variance (3 study groups).

Outcome measure	Interaction effect (outcome time & group)	Within-subjects: time effect	Between-subjects: group effect
TS trapezius	***p*= 0.003**	*p*= NA	*p*= NA
CPM trapezius	*p*= 0.755	***p*= 0.002**	*p*= 0.639

TS: temporal summation, CPM: conditioned pain modulation, NA: not applicable

Table 3 Contrasts: Repeated measures analysis of variance (3 study groups).

Within-subjects contrasts (simple first)	After relaxation vs. baseline mean	After cognition vs. baseline mean
TS trapezius*group	***p*= 0.008**	***p*= 0.003**
CPM trapezius	***p*= 0.002**	***p*= 0.001**

TS: temporal summation, CPM: conditioned pain modulation

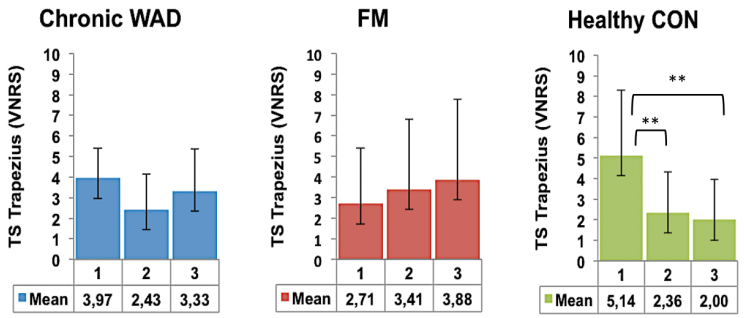


Figure 2 Effects of relaxation and cognitive stress on TS in patients with chronic WAD, FM and healthy controls.

Values are presented as mean and confidence interval. TS: temporal summation, VNRS: verbal numeric rating scale, WAD: whiplash-associated disorders, FM: fibromyalgia, **= $p < 0.01$

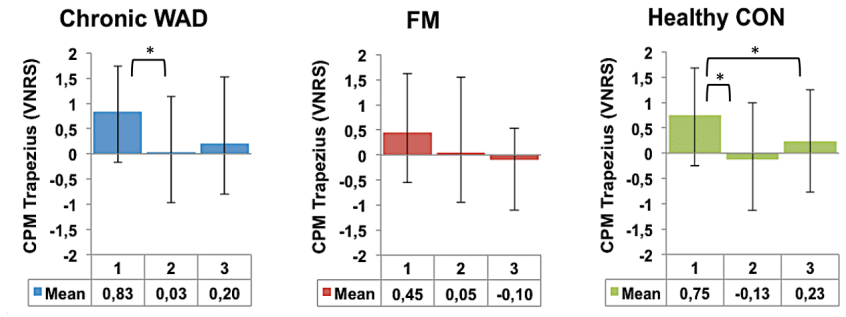


Figure 3 Effects of relaxation and cognitive stress on CPM efficacy in patients with chronic WAD, FM and healthy controls.

Values are presented as mean and confidence interval. CPM: conditioned pain modulation, VNRS: verbal numeric rating scale, WAD: whiplash-associated disorders, FM: fibromyalgia, *= $p < 0.05$

Discussion

The present study is the first to examine the effects of a single relaxation session and a cognitive stressor on central pain modulation in chronic WAD and FM patients compared to healthy individuals. The study results indicate that both types of interventions enhance TS of pain in FM, indicating an increased nociceptive transmission to the brain in these patients (bottom-up sensitization). In contrast, chronic WAD patients and healthy controls experienced acute positive effects on bottom-up sensitization, as both relaxation and cognitive stress reduced TS of pain. However, both interventions resulted in decreased CPM efficacy in healthy people as well as in those suffering from chronic pain (chronic WAD and FM), indicating that they have a detrimental effect on endogenous pain inhibition.

Possibly, performing the cognitive tasks served as a high cognitive stressor for FM patients, already suffering attention and memory problems [62,63], which further burdened the central nervous system leading to further disinhibition and more pain (self-reported hyperalgesia). In line with these results, Crettaz and colleagues [28] reported enhanced pain sensitivity to pressure stimuli in FM patients, but not in healthy participants following psychological stress, induced using the Trier Social stress test. Stress-induced hyperalgesia has been demonstrated in FM in other studies as well [64,65]. However, these studies have not investigated the effect of stress on TS and CPM.

In contrast, unpublished study results show that chronic WAD patients encounter less attention and memory problems than FM patients [66]. Therefore, it can be hypothesized that chronic WAD patients and healthy individuals may have experienced the cognitive tests as less challenging and less stressful. Accordingly, this could have led to decreased nociceptive ascending transmission, so diminished TS. Indeed, previous researchers have reported that the perceived severity of the stressor can influence pain modulation [26].

Secondly, it is possible that the cognitive tasks diminished pain sensitivity as a result of the previously mentioned "distraction effect" [45]. This may be the explanation in the chronic WAD and control group, since TS of pain was reduced after the cognitive stressor.

Regarding the PRT, the format of the relaxation may have served as a physical stressor in the FM group, but to a lesser extent or not in the chronic WAD and control groups. This relaxation technique requires alternate tightening and relaxing of different muscle groups aimed at decreasing overall muscle tension [67]. In chronic WAD and FM, PRT has the advantage of emphasizing the difference between muscles that are relaxed and those that are tensed, since a subgroup of patients continuously tense their muscles which can contribute to persistent pain [68]. However, it may be that some FM patients tensed their (already painful) muscles too tightly and focused their attention even more on the pain during the relaxation session, which could have resulted in exacerbating pain and enhanced TS. There is one controlled trial in which the effects of biofeedback PRT using surface EMG were compared with the effects of a fitness program or a usual-care treatment (6 months) [69]. PRT was indeed not effective in reducing pain perception or psychological distress, including stress and anxiety levels [69]. Recently, a systematic review regarding the effects of relaxation therapy on pain also showed limited evidence supporting the use of muscle relaxation as a sole treatment for reducing pain in FM patients [43]. The authors suggested that PRT was not effective as a standalone treatment strategy but that it could possibly improve pain relief when used in combination with other strategies, such as exercise and guided imagery.

In line with our results of diminished TS after the PRT in healthy individuals, Emery et al [70] found an increased nociceptive flexion reflex threshold, which is an objective indication for reduced central hyperexcitability, after a single progressive relaxation session in healthy pain-free adults.

Interesting, this study found decreased CPM efficacy following PRT and a cognitive stressor in patients with chronic WAD and FM as well as in healthy persons. Previous work has shown that CPM responses depend on the interplay between physical and psychological mechanisms [71], influenced by cognitive factors, including attention, distraction, and expectations. It is possible that adequate CPM activation after the interventions was affected by these factors. Additionally, it could be that before a second CPM activation, a recovery period is needed after a previous CPM activation. Previous studies have also demonstrated worsened CPM responses following a second CPM measurement [72,73]. Therefore, it may be that each successive conditioned noxious stimulus decreases CPM efficacy.

Kristian et al [73] investigated the effect of a simple mental stressor (mathematical calculations) and a non-stressful intervention (passive listening to a tale for children) on CPM of heat pain in healthy participants. They found a reduced CPM effect following the stressful as well as the non-stressful intervention, which is in line with our observations.

Limitations, strengths, and suggestions for further research

The present crossover study has a few study limitations that have to be taken into consideration. First, the obtained results only apply for a single session of muscle relaxation therapy and cognitive stress, whereby no conclusions can be made for effects on pain perception and modulation of chronic cognitive stress and long-term relaxation therapies. Second, the number of study participants (n=59) was rather small, whereby bigger samples in each group could have provided more generalizable results. Thirdly, the use of antidepressiva was significantly different between FM patients and healthy persons. Fourthly, the results of the CPM measures are characterized by wide confidence intervals. However, the variance for CPM of the 3 study groups was not significantly different (p>0.05). At last, autonomic variables, anxiety, and individuals' perception of stress were not measured in this study. Therefore, future protocols could adjust for these variables and include the assessment of the individual's perceived level of mental stress during the stressful and non-stressful task. Measurement of cortisol and catecholamine levels could give valuable information on the perceived level of stress.

Despite these limitations, the current study also has important strengths. First, the used randomized longitudinal crossover design, in which all participants are exposed to both tasks and serve as their own controls, minimizes bias and variability. Second, sources of bias like medication use were anticipated and well defined diagnostic criteria were utilized for chronic WAD and FM. Finally, this paper adds relevant knowledge to the current literature regarding stress-pain and relaxation-pain interactions in patients with chronic WAD and FM.

Future studies are warranted to help further elucidate the complex relation between stress, relaxation, and pain, and the involved underlying mechanisms. It could be interesting to examine the effects of other relaxation techniques like mindfulness, yoga, mind-body exercises, or visualization on pain modulation in patients with CS pain.

Further, inclusion of EMG biofeedback would provide a more accurate assessment of actual muscle relaxation.

The current study obtained new insight in the effect of acute stress and relaxation on central pain modulation in the investigated population. The unravelling of influencing factors on central pain modulation is in our opinion an important first step in order to adapt future interventions for chronic pain patients adequately.

It remains an important challenge for researchers and therapists to develop effective therapy strategies for chronic pain patients characterized by CS.

Clinical implications

Based on the present results, it can be summarized that acute experimental psychophysical stress due to the aforementioned interventions can lead to decreased efficacy of pain modulation, especially in patients with FM. Noteworthy is that cognitive stress exerted a similar influence on pain modulation as a PRT session.

Therapists should be aware of the possible negative and/or positive influences of cognitive demanding tasks and relaxation techniques which depend on body and or muscle movements on pain modulation, depending on the patient's individual ability to cope with stress. By assessing and questioning patients, the nature of the effect can become clear and the program can be adapted when needed. Measuring pain sensitivity and the perceived level of stress following a stressor is valuable for identifying patients that have problems with their stress-response system.

Taken together, we suggest a multicomponent assessment and rehabilitation in which the underlying pathophysiological mechanisms should be taken into account.

Conclusion

In FM, one session of PRT and cognitive stress exaggerated TS, hence increased nociceptive transmission to the brain. Therefore, it can be assumed that a single relaxation session as well as cognitive stress may have negative acute effects on pain modulation in patients with FM, while cognitive stress and relaxation reduced TS in both chronic WAD patients and healthy controls. Lower TS values point towards reduced bottom-up sensitization, possibly due to a change in brain focus as a result of distraction.

Endogenous pain inhibition, measured with the CPM paradigm, worsened in response to both relaxation and cognitive stress in healthy people, chronic WAD patients, and FM patients.

These results should be taken into consideration when developing therapy strategies for patients with chronic WAD and FM.

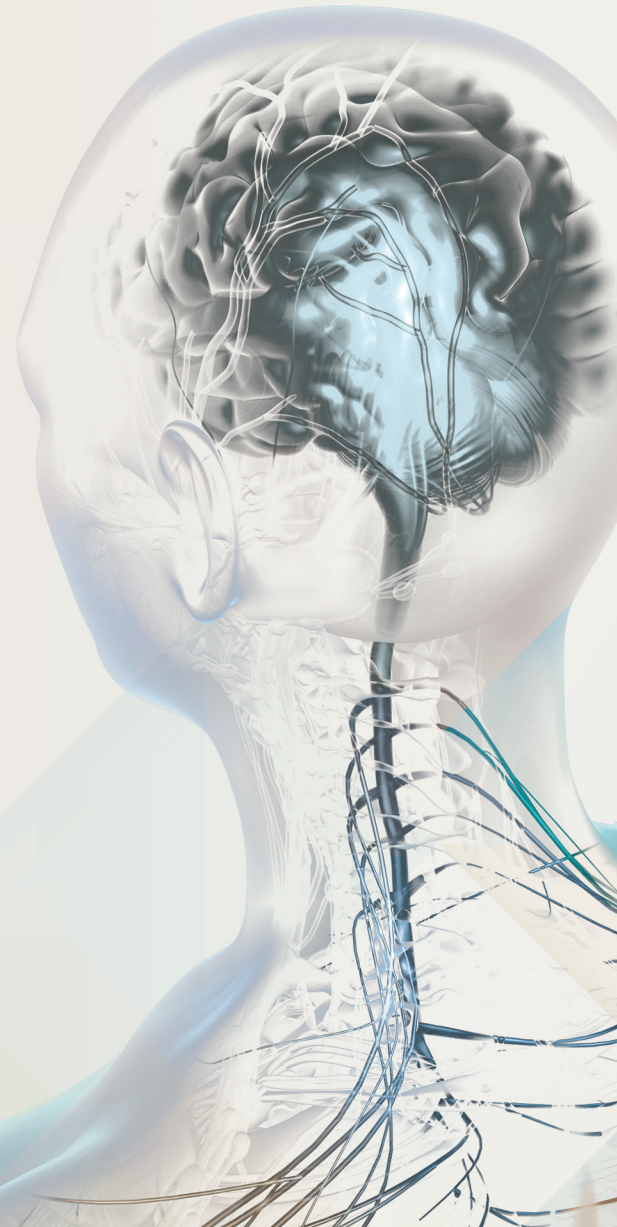
References

1. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *European journal of pain (London, England)* 2013:17: 299-312.
2. Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clinical rheumatology* 2007:26: 465-473.
3. Yunus MB. Role of central sensitization in symptoms beyond muscle pain, and the evaluation of a patient with widespread pain. *Best practice & research Clinical rheumatology* 2007:21: 481-497.
4. Staud R, Smitherman ML. Peripheral and central sensitization in fibromyalgia: pathogenetic role. *Current pain and headache reports* 2002:6: 259-266.
5. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Manual therapy* 2010:15: 135-141.
6. Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, Curatolo M. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. *Pain* 2004:107: 7-15.
7. Meeus M, Nijs J, Van de Wauwer N, Toeback L, Truijen S. Diffuse noxious inhibitory control is delayed in chronic fatigue syndrome: an experimental study. *Pain* 2008:139: 439-448.
8. Desmeules JA, Cedraschi C, Rapiti E, Baumgartner E, Finckh A, Cohen P, Dayer P, Vischer TL. Neurophysiologic evidence for a central sensitization in patients with fibromyalgia. *Arthritis and rheumatism* 2003:48: 1420-1429.
9. Neziri AY, Haesler S, Petersen-Felix S, Muller M, Arendt-Nielsen L, Manresa JB, Andersen OK, Curatolo M. Generalized expansion of nociceptive reflex receptive fields in chronic pain patients. *Pain* 2010:151: 798-805.
10. Kaya S, Hermans L, Willems T, Roussel N, Meeus M. Central sensitization in urogynecological chronic pelvic pain: a systematic literature review. *Pain physician* 2013:16: 291-308.
11. Bouhassira D, Gall O, Chitour D, Le Bars D. Dorsal horn convergent neurones: negative feedback triggered by spatial summation of nociceptive afferents. *Pain* 1995:62: 195-200.
12. Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, Hansson P, Lautenbacher S, Marchand S, Wilder-Smith O. Recommendations on terminology and practice of psychophysical DNIC testing. *European journal of pain (London, England)* 2010:14: 339.
13. Zusman M. Forebrain-mediated sensitization of central pain pathways: 'non-specific' pain and a new image for MT. *Manual therapy* 2002:7: 80-88.
14. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, Vierck CJ. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. *Pain* 2002:99: 49-59.
15. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Giani C, Zbinden AM, Radanov BP. Central hypersensitivity in chronic pain after whiplash injury. *The Clinical journal of pain* 2001:17: 306-315.
16. Lemming D, Sorensen J, Graven-Nielsen T, Arendt-Nielsen L, Gerdle B. The responses to pharmacological challenges and experimental pain in patients with chronic whiplash-associated pain. *The Clinical journal of pain* 2005:21: 412-421.
17. Vierck CJ, Jr., Staud R, Price DD, Cannon RL, Mauderli AP, Martin AD. The effect of maximal exercise on temporal summation of second pain (windup) in patients with fibromyalgia syndrome. *The journal of pain : official journal of the American Pain Society* 2001:2: 334-344.
18. Van Oosterwijck J, Nijs J, Meeus M, Van Loo M, Paul L. Lack of endogenous pain inhibition during exercise in people with chronic whiplash associated disorders: an experimental study. *The journal of pain : official journal of the American Pain Society* 2012:13: 242-254.
19. Lannersten L, Kosek E. Dysfunction of endogenous pain inhibition during exercise with painful muscles in patients with shoulder myalgia and fibromyalgia. *Pain* 2010:151: 77-86.
20. Kasch H, Qerama E, Bach FW, Jensen TS. Reduced cold pressor pain tolerance in non-recovered whiplash patients: a 1-year prospective study. *European journal of pain (London, England)* 2005:9: 561-569.
21. Julien N, Goffaux P, Arsenault P, Marchand S. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain* 2005:114: 295-302.
22. Crofford LJ. The hypothalamic-pituitary-adrenal stress axis in fibromyalgia and chronic fatigue syndrome. *Zeitschrift fur Rheumatologie* 1998:57 Suppl 2: 67-71.

23. Gaab J, Baumann S, Budnoik A, Gmunder H, Hottinger N, Ehlert U. Reduced reactivity and enhanced negative feedback sensitivity of the hypothalamus-pituitary-adrenal axis in chronic whiplash-associated disorder. *Pain* 2005:119: 219-224.
24. Kuehl LK, Michaux GP, Richter S, Schachinger H, Anton F. Increased basal mechanical pain sensitivity but decreased perceptual wind-up in a human model of relative hypocortisolism. *Pain* 2010:149: 539-546.
25. Butler RK, Finn DP. Stress-induced analgesia. *Progress in neurobiology* 2009:88: 184-202.
26. Olango WM, Finn DP. Neurobiology of stress-induced hyperalgesia. *Current topics in behavioral neurosciences* 2014:20: 251-280.
27. Cathcart S, Petkov J, Pritchard D. Effects of induced stress on experimental pain sensitivity in chronic tension-type headache sufferers. *European journal of neurology : the official journal of the European Federation of Neurological Societies* 2008:15: 552-558.
28. Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, Burgmer M. Stress-induced allodynia--evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. *PloS one* 2013:8: e69460.
29. Martenson ME, Cetas JS, Heinricher MM. A possible neural basis for stress-induced hyperalgesia. *Pain* 2009:142: 236-244.
30. Koltyn KF, Arbogast RW. Perception of pain after resistance exercise. *British journal of sports medicine* 1998:32: 20-24.
31. Koltyn KF. Analgesia following exercise: a review. *Sports medicine (Auckland, NZ)* 2000:29: 85-98.
32. Millan MJ. Descending control of pain. *Progress in neurobiology* 2002:66: 355-474.
33. Ray CA, Carter JR. Central modulation of exercise-induced muscle pain in humans. *The Journal of physiology* 2007:585: 287-294.
34. Jennings EM, Okine BN, Roche M, Finn DP. Stress-induced hyperalgesia. *Progress in neurobiology* 2014:121: 1-18.
35. Spitzer WO, Skovron ML, Salmi LR, Cassidy JD, Duranceau J, Suissa S, Zeiss E. Scientific monograph of the Quebec Task Force on Whiplash-Associated Disorders: redefining "whiplash" and its management. *Spine* 1995:20: 1s-73s.
36. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis and rheumatism* 1990:33: 160-172.
37. Seo J, Kim SH, Kim YT, Song HJ, Lee JJ, Kim SH, Han SW, Nam EJ, Kim SK, Lee HJ, Lee SJ, Chang Y. Working memory impairment in fibromyalgia patients associated with altered frontoparietal memory network. *PloS one* 2012:7: e37808.
38. Nederhof E, Lemmink KA, Visscher C, Meeusen R, Mulder T. Psychomotor speed: possibly a new marker for overtraining syndrome. *Sports medicine (Auckland, NZ)* 2006:36: 817-828.
39. Grace GM, Nielson WR, Hopkins M, Berg MA. Concentration and memory deficits in patients with fibromyalgia syndrome. *Journal of clinical and experimental neuropsychology* 1999:21: 477-487.
40. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. *Progress in neurobiology* 2011:93: 385-404.
41. Antepohl W, Kiviloog L, Andersson J, Gerdle B. Cognitive impairment in patients with chronic whiplash-associated disorder--a matched control study. *NeuroRehabilitation* 2003:18: 307-315.
42. Weiner DK, Rudy TE, Morrow L, Slaboda J, Lieber S. The relationship between pain, neuropsychological performance, and physical function in community-dwelling older adults with chronic low back pain. *Pain medicine (Malden, Mass)* 2006:7: 60-70.
43. Meeus M, Nijs J, Vanderheiden T, Baert I, Descheemaeker F, Struyf F. The effect of relaxation therapy on autonomic functioning, symptoms and daily functioning, in patients with chronic fatigue syndrome or fibromyalgia: a systematic review. *Clinical rehabilitation* 2014.
44. E J. Electrical measurements concerning muscular contraction (tonus) and the cultivation of relaxation in man: relaxation times of individuals. . *American Journal of Physiology* 1934a:108: 573–580.
45. Eccleston C, Crombez G. Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychological bulletin* 1999:125: 356-366.
46. FJ. M. Progressive relaxation: origins, principles, and clinical applications. Principles and practice of stress management. edition. Guilford Press, New York, 1993.
47. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis care & research* 2010:62: 600-610.
48. Cathcart S, Winefield AH, Rolan P, Lushington K. Reliability of temporal summation and diffuse noxious inhibitory control. Pain research & management: the journal of the Canadian Pain Society = journal de la societe canadienne pour le traitement de la douleur 2009:14: 433-438.
49. Staud R, Robinson ME, Price DD. Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. *The journal of pain : official journal of the American Pain Society* 2007:8: 893-901.
50. Meeus M, Nijs J, Huybrechts S, Truijen S. Evidence for generalized hyperalgesia in chronic fatigue syndrome: a case control study. *Clinical rheumatology* 2010:29: 393-398.
51. Fernandez-Carnero J, Fernandez-de-Las-Penas C, de la Llave-Rincon AI, Ge HY, Arendt-Nielsen L. Widespread mechanical pain hypersensitivity as sign of central sensitization in unilateral epicondylalgia: a blinded, controlled study. *The Clinical journal of pain* 2009:25: 555-561.
52. Kosek E, Ekholm J, Hansson P. Pressure pain thresholds in different tissues in one body region. The influence of skin sensitivity in pressure algometry. *Scandinavian journal of rehabilitation medicine* 1999:31: 89-93.
53. Vanderweeen L, Oostendorp RA, Vaes P, Duquet W. Pressure algometry in manual therapy. *Manual therapy* 1996:1: 258-265.
54. Antonaci F, Sand T, Lucas GA. Pressure algometry in healthy subjects: inter-examiner variability. *Scandinavian journal of rehabilitation medicine* 1998:30: 3-8.
55. Daenen L, Nijs J, Roussel N, Wouters K, Van Loo M, Cras P. Dysfunctional pain inhibition in patients with chronic whiplash-associated disorders: an experimental study. *Clinical rheumatology* 2013:32: 23-31.
56. De Kooning M, Daenen L, Cras P, Gidron Y, Roussel N, Nijs J. Autonomic response to pain in patients with chronic whiplash associated disorders. *Pain physician* 2013:16: E277-285.
57. Meeus M, Ickmans K, Struyf F, Hermans L, Van Noesel K, Oderkerk J, Declerck LS, Moorkens G, Hans G, Grosemans S, Nijs J. Does acetaminophen activate endogenous pain inhibition in chronic fatigue syndrome/fibromyalgia and rheumatoid arthritis? A double-blind randomized controlled cross-over trial. *Pain physician* 2013:16: E61-70.
58. E. J. Electrical measurements concerning muscular contraction (tonus) and the cultivation of relaxation in man: studies on arm flexors. American Journal of Physiology. 1934b:107: 230–248.
59. Ickmans K, Clarys P, Nijs J, Meeus M, Aerenhouts D, Zinzen E, Aelbrecht S, Meersdom G, Lambrecht L, Pattyn N. Association between cognitive performance, physical fitness, and physical activity level in women with chronic fatigue syndrome. *Journal of rehabilitation research and development* 2013:50: 795-810.
60. Ickmans K, Meeus M, Kos D, Clarys P, Meersdom G, Lambrecht L, Pattyn N, Nijs J. Cognitive performance is of clinical importance, but is unrelated to pain severity in women with chronic fatigue syndrome. *Clinical rheumatology* 2013:32: 1475-1485.
61. Meeus M, Van Oosterwijck J, Ickmans K, Baert I, Coppieters I, Roussel N, Struyf F, Pattyn N, Nijs J. Interrelationships between pain processing, cortisol and cognitive performance in chronic whiplash-associated disorders. *Clinical rheumatology* 2013.
62. Tesio V, Torta DM, Colonna F, Leombruni P, Ghiggia A, Fusaro E, Geminiani GC, Torta R, Castelli L. Are fibromyalgia patients cognitively impaired? Objective and subjective neuropsychological evidence. *Arthritis care & research* 2015:67: 143-150.
63. Suhr JA. Neuropsychological impairment in fibromyalgia: relation to depression, fatigue, and pain. *Journal of psychosomatic research* 2003:55: 321-329.
64. Van Houdenhove B, Luyten P. Stress, depression and fibromyalgia. *Acta neurologica Belgica* 2006:106: 149-156.
65. Nilsen KB, Sand T, Westgaard RH, Stovner LJ, White LR, Bang Leistad R, Helde G, Ro M. Autonomic activation and pain in response to low-grade mental stress in fibromyalgia and shoulder/neck pain patients. *European journal of pain (London, England)* 2007:11: 743-755.
66. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive performance is related to central sensitization and health-related quality of life in patients with chronic whiplash-associated disorders and fibromyalgia. *Pain physician* 2015:18.
67. Jacobson E. *Progressive relaxation*. University of Chicago Press, Chicago, 1938.

68. Elert J, Kendall SA, Larsson B, Mansson B, Gerdle B. Chronic pain and difficulty in relaxing postural muscles in patients with fibromyalgia and chronic whiplash associated disorders. *The Journal of rheumatology* 2001:28: 1361-1368.
69. van Santen M, Bolwijn P, Verstappen F, Bakker C, Hidding A, Houben H, van der Heijde D, Landewe R, van der Linden S. A randomized clinical trial comparing fitness and biofeedback training versus basic treatment in patients with fibromyalgia. *The Journal of rheumatology* 2002:29: 575-581.
70. Emery CF, France CR, Harris J, Norman G, Vanarsdalen C. Effects of progressive muscle relaxation training on nociceptive flexion reflex threshold in healthy young adults: a randomized trial. *Pain* 2008:138: 375-379.
71. Nir RR, Yarnitsky D, Honigman L, Granot M. Cognitive manipulation targeted at decreasing the conditioning pain perception reduces the efficacy of conditioned pain modulation. *Pain* 2012:153: 170-176.
72. Meeus M, Hermans L, Ickmans K, Struyf F, Van Cauwenbergh D, Bronckaerts L, De Clerck LS, Moorken G, Hans G, Grosemans S, Nijs J. Endogenous Pain Modulation in Response to Exercise in Patients with Rheumatoid Arthritis, Patients with Chronic Fatigue Syndrome and Comorbid Fibromyalgia, and Healthy Controls: A Double-Blind Randomized Controlled Trial. *Pain practice* 2014.
73. Kristian B. Nilsen SEC, Line B. Holmen, Trond Sand. The effect of a mental stressor on conditioned pain modulation in healthy subjects. *Scandinavian Journal of Pain* 2012:3: 142-148.

General Discussion



General Discussion

Summary and discussion of the results

The **core aim** of this dissertation was to improve our knowledge of the mechanisms and their possible interrelationships underlying the persistent and often unexplained symptoms in patients with chronic idiopathic neck pain (INP), chronic whiplash associated disorders (WAD), and fibromyalgia (FM). The core aim was furthermore to unravel differences in the nature and severity of the underpinning mechanisms between these three heterogeneous conditions with chronic musculoskeletal pain. This way, the present thesis aimed to further disentangle pieces of the complex pathophysiological puzzle underlying chronic INP, chronic WAD, and FM with the ultimate endeavor to increase our understanding of these conditions. The present dissertation was composed of three major parts, each addressing one overall objective. In the following paragraphs the most important results of each part will be summarized and discussed.

Part I: Is chronic musculoskeletal pain associated with structural and functional brain alterations?

No clear overview of how brain alterations are related to clinical correlates of pain in various chronic musculoskeletal pain conditions was present. However, knowledge on this relationship is important to unravel the underlying mechanisms of persistent pain. Therefore, we performed a systematic review, described in *chapter 1,* to investigate relationships between brain alterations and clinical pain measures in patients with chronic musculoskeletal pain. In line with our hypothesis, most studies revealed significant **relationships** between structural or functional **brain alterations**, and **clinical pain correlates** encompassing pain intensity, unpleasantness, pain duration, sensory hypersensitivity, and pressure pain sensitivity. Specifically, moderate evidence was found for relationships between clinical pain measures and alterations in grey matter (GM) morphology (volume and thickness), white matter (WM) microstructure (fractional anisotropy and axial diffusivity), and (resting-state) functional connectivity in **brain regions or networks** involved in **somatosensory, affective-motivational**, and **cognitive pain processing** in patients with chronic musculoskeletal pain. The evidence regarding the direction of these relationships (e.g., increased or decreased GM volume associated with more severe clinical pain measures) was inconclusive. Noteworthy, the direction and nature of the relationships between clinical pain characteristics and brain alterations could have been influenced by multiple factors which possibly explains the observed incongruent results. These factors could be the variety of patient conditions that are classified as chronic musculoskeletal pain, and the different scales or questionnaires that have been used to measure clinical features of pain. Furthermore, the various MRI acquisition and analyzing techniques as well as the investigated brain regions could have influenced the exposed relationships. Also, research has demonstrated the influence of

age, sex, preexisting vulnerabilities, genetics, previous experiences, use of medications, and psychosocial factors on pain and neuroplasticity in patients with chronic pain [1-11].

In summary, different chronic musculoskeletal pain syndromes exposed unique (specific for each pain syndrome) anatomical and functional brain alterations or "brain signatures". However, it appeared that brain regions implicated in motivational-affective and cognitive components of pain processing were involved in the observed neuroplasticity in all these pain conditions. Based on this compelling evidence, we can infer that chronic musculoskeletal pain is not only mediated by somatosensory processing of pain but there is a **shift towards** critical involvement of **cognitive** and **motivational-affective pain processing** in regions often part of the limbic system, such as the anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), hippocampus, insula, orbitofrontal cortex, and amygdala. Furthermore, it appeared that brain alterations remained unexplored in patients with chronic INP and chronic WAD. As such, before performing brain MRI research in these patients, the need arose to systematically address the evidence of brain alterations in different neck pain patients, examined by a wider range of medical brain imaging techniques. Consequently, we conducted a systematic review, enclosed in *chapter 2*, aimed at reviewing the evidence of structural or functional brain alterations in patients with acute, subacute, or chronic non-traumatic INP and traumatic neck pain (WAD), examined with brain MRI techniques, or Positron Emission Tomography, or Single-Photon Emission Computed Tomography.

Based on a paucity of studies, moderate evidence was found for the absence of structural brain abnormalities in the acute phase after a whiplash injury. [12, 13]. In contrast, in **chronic WAD** patients encountering persistent post-traumatic headache, **decreased GM volume** in the ACC and the dorsolateral prefrontal cortex was revealed [14]. The ACC and dorsolateral prefrontal cortex are involved in the salience and affective-cognitive dimensions of pain, and play an important role in top-down pain inhibition [15-17]. On the contrary, **increased GM volume** was demonstrated in the periaqueductal grey, thalamus, and cerebellum in these chronic WAD patients [14]. Interestingly, the periaqueductal grey is crucially involved in endogenous pain inhibition [18]. It has been suggested that the observed structural neuroplasticity (increased and decreased GM morphology) might result from the aim to **restore the balance** between nociceptive (i.e. **pain facilitation**) and anti-nociceptive (i.e. **pain inhibition**) modulation [14].

With respect to functional brain alterations, some evidence was found for decreased brain activation in temporal regions during a visual task in patients with chronic WAD compared to controls [19]. Furthermore, some evidence was revealed for alterations in brain **neurotransmission** encompassing decreased neurokinin 1 receptor availability in patients with **chronic WAD** compared to controls [20]. Interestingly, these receptors are involved in pain processing [21] and central sensitization (CS) [22]. In addition, several studies observed alterations in **brain perfusion** and associations with self-reported pain and

related disability were found [23, 24]. Specifically, both increased and decreased regional cerebral blood flow were demonstrated in patients with **chronic INP** and **chronic WAD** compared to healthy controls [23-28]. A higher amount of regional cerebral blood flow might reflect a compensation mechanism for regional brain atrophy [29], and the variety of brain perfusion abnormalities might correspond with the observed diversity in structural brain changes in patients with chronic WAD [28].

To summarize, some to moderate evidence was found for structural and functional brain alterations in patients with chronic WAD, and only for functional brain alterations in patients with chronic INP [30]. More evidence exists for brain alterations in patients with chronic WAD relative to patients with chronic INP, suggesting the presence of different underlying mechanisms in both populations. Moreover, the diversity in observed brain alterations indicates that various mechanisms are responsible for the brain's neuroplasticity associated with chronic neck pain. Accordingly, brain alterations and relationships with clinical measures should be further investigated in chronic INP and chronic WAD patients with more sophisticated and sensitive brain imaging techniques. Therefore, structural brain alterations and relationships with clinical measures were examined in women with chronic INP and chronic WAD in the original research studies enclosed in *chapters 4* and *5*.

Part II: Differences between idiopathic and traumatic chronic neck pain: interrelationships among disability, cognitive deficits, central sensitization, and structural brain alterations.

In **part II**, three original research studies were described approaching the **second overall objective** of this dissertation to gain insight in possible differences between women with chronic neck pain of traumatic and non-traumatic idiopathic origin. We hypothesized that the traumatic nature of neck pain in patients with chronic WAD would be associated with more severe deficits compared to patients with chronic INP.

As can be seen in **table 1** and **figures 1** and **2**, the research findings of *chapters 3 to 5* indicated that women with chronic INP and chronic WAD are characterized by some similarities but also prominent differences in the assessed clinical variables and structural brain measures as well as differences in their interrelationships were found, revealing **different underlying mechanisms**. Consistent with our hypothesis, patients with chronic WAD showed more severe deficits than patients with chronic INP. In particular, pain-related disability, decreased health-related quality of life, and self-perceived cognitive deficits were demonstrated in women with chronic WAD and, to a significantly lesser extent in women with chronic INP [31]. Furthermore, both patient groups reported higher levels of CS symptoms compared to healthy controls, however, only in the chronic WAD group these self-perceived symptoms were indicative of CS (central sensitization inventory (CSI) score ≥ 40 of 100). In addition, decreased objective cognitive performance and

higher levels of pain catastrophizing and hypervigilance were present only in women with chronic WAD. In addition, distant hyperalgesia and decreased efficacy of conditioned pain modulation (CPM) were shown in chronic WAD but not in chronic INP patients, indicating the presence of CS only in the participants with chronic WAD at a group level.

This study furthermore revealed relationships among higher disability, more severe subjective and objective cognitive deficits, and more extensive local and distant hyperalgesia in women with chronic WAD. However, in women with chronic INP, relationships were found only between subjective cognitive deficits and local hyperalgesia. In contrast to our hypothesis, decreased CPM efficacy or dysfunctional endogenous pain inhibition was not associated with decreased cognitive performance. Yet, the study enclosed in *chapter 6* also did not find significant correlations between decreased cognitive performance and CPM efficacy in patients with chronic WAD [32].

Table 1 Summary of the differences revealed between women with chronic INP and women with chronic WAD, and compared to healthy pain-free women in the studies enclosed in part II.

Features that were demonstrated only in chronic WAD and not in chronic INP patients
Observed only in chronic WAD
Objective cognitive deficits
Experimental signs of CS encompassing distant hyperalgesia and dysfunctional CPM
Higher levels of pain catastrophizing and hypervigilance
Decreased GM volume in the left PCC, the right superior parietal cortex, the right lateral orbitofrontal cortex, the left supramarginal cortex
Cortical thinning in the left precuneus
Abnormalities in WM microstructure in the left cingulum hippocampus and the left tapetum
Features that were present in chronic INP and chronic WAD patients but to a lesser extent in patients with chronic INP
Chronic WAD > chronic INP
Subjective cognitive deficits
Current neck pain intensity
Pain-related disability
Limitations on health-related quality of life
Self-reported symptoms of CS

The above outlined findings provided insight in one piece of the puzzle for understanding chronic INP and chronic WAD, and their differences. However, we were convinced that another part of the puzzle for disentangling the underlying mechanisms could be uncovered by performing brain MRI research in these patients.

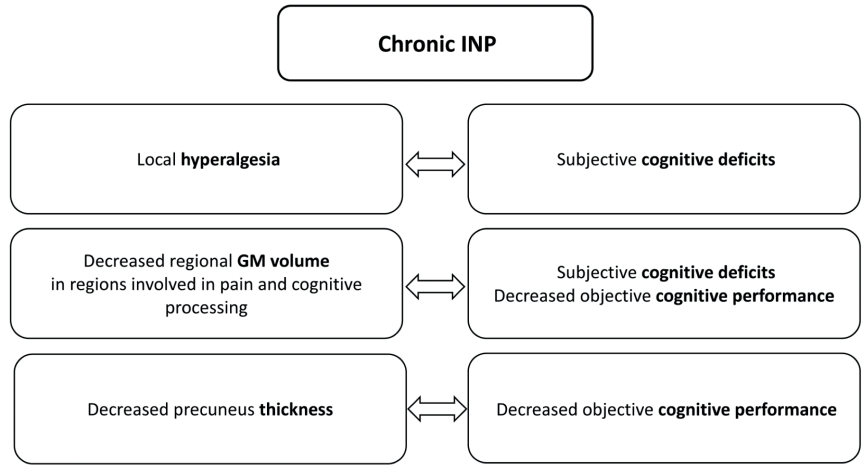


Figure 1 Overview of the observed relationships between the investigated underlying mechanisms in women with chronic INP.

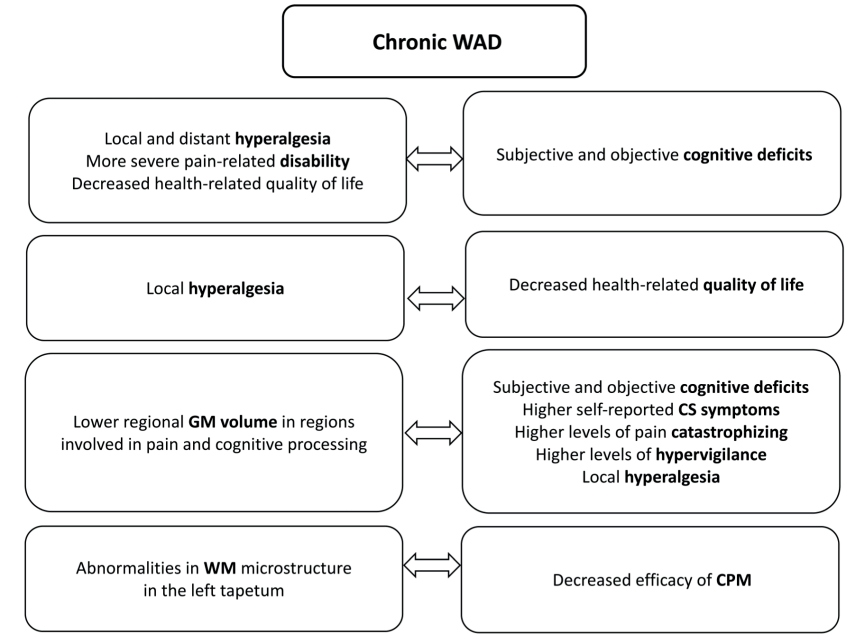


Figure 2 Overview of the observed relationships between the investigated underlying mechanisms in women with chronic WAD.

The results of the case-control studies described in *chapters 4 and 5* provided evidence of **decreased GM volume** in cortical regions associated with pain and cognitive processing in women with chronic WAD compared to women with chronic INP and healthy women. These results were in line with our hypothesis and could maybe be explained because chronic WAD patients have a traumatic origin of neck pain and are characterized by CS in contrast to patients with non-traumatic chronic INP. Furthermore, these findings are in line with accumulating evidence of decreased regional GM volume in other chronic pain populations sharing the common pathophysiology of CS such as FM and chronic low back pain compared to healthy persons [33-37]. Additionally, in women with chronic WAD, decreased GM volume in cognitive and pain processing regions was associated with increased cognitive deficits, maladaptive pain cognitions, self-reported CS symptoms, and local hyperalgesia. In women with chronic INP, decreased regional GM volume was only associated with increased cognitive deficits but compared with healthy controls, no GM volume alterations could be revealed.

Furthermore, **cortical thinning** in the left precuneus was found in women with chronic WAD compared to women with chronic INP. This result is interesting because the precuneus is part of the structural brain core [38] and a core hub (i.e. highly interconnected nodes) of the default mode network [39]. In addition, the precuneus plays a crucial role in a wide range of cognitive and mental processes [40]. In contrast to the results of previous studies in patients with chronic musculoskeletal pain [34, 41] and mild traumatic brain injury (TBI) [42], differences in cortical thickness between patients with chronic WAD and healthy controls could not be demonstrated. The latter result is in contrast with the findings of decreased regional GM volume in patients with chronic WAD compared to healthy controls. Nevertheless, altered GM volume in the precuneus was not observed, highlighting that cortical thickness and GM volume reflect different features of the underlying neural architecture. Surface area, curvature, and grey/white matter intensity contrast could account for some differences between GM volume and cortical thickness [43]. Additionally, unlike regional GM volume, cortical thinning in the left precuneus was not associated with worse cognitive performance or measures of CS in patients with chronic WAD. On the contrary, in chronic INP patients, decreased precuneus thickness coincided with worse performance on the trail making test (TMT) part B, however compared to controls, decreased cortical thickness and worse objective cognitive performance could not be revealed.

In addition, **abnormalities** in **WM microstructure** were revealed in two WM tracts carrying information between regions involved in pain and cognitive processing in women with chronic WAD compared to women with chronic INP or healthy women. The observed WM microstructural alterations in patients with chronic WAD were in line with our hypothesis. Specifically, results showed a consistent pattern of decreased fractional anisotropy coinciding with increased mean diffusivity and increased radial diffusivity in the left cingulum hippocampus and in the left tapetum in patients with chronic WAD

compared to healthy controls (tapetum) or compared to patients with chronic INP (cingulum and tapetum). We can very carefully suggest that maybe to some extent these findings reflect WM demyelination in these tracts evidenced by the unchanged axial diffusivity together with increased radial diffusivity in women with chronic WAD [44-46]. The revealed pattern of WM microstructural deficits in chronic WAD is consistent with the results in other chronic musculoskeletal pain conditions [47]. Furthermore, this study provided evidence for **associations** between **dysfunctional CPM** and the degree of WM deficits in the left tapetum in patients with chronic WAD. This demonstrates innovative evidence for underlying WM microstructural correlates of disturbed endogenous pain inhibition in patients with chronic WAD. Accordingly, the results of part II emphasize a pathophysiological role of **structural brain alterations** in patients with **chronic WAD** compared to chronic INP patients, possibly mediated by the whiplash injury, CS, and more severe cognitive deficits.

Interestingly, brain microhemorrhages related to trauma or diffuse axonal injury were not observed in all study groups. Previous studies could detect microhemorrhagic lesions in the brain in patients with mild TBI suggesting differences in underlying pathophysiological mechanisms between patients with chronic WAD and mild TBI [48]. To our knowledge this study is the first to assess the presence of brain microhemorrhages in patients with chronic WAD.

Based on the findings of *chapter 3*, we believed it was clinically relevant to further explore the presence of CS in our chronic INP and chronic WAD populations, thus we performed **additional data analyses** including the study sample of *chapter 3*. The results of these analyses are added to the present dissertation as additional research data in appendix. To date, efficacy of treatment response is variable in both conditions, which possibly originates from the focus on the diagnostic vignette when treating patients with chronic INP or chronic WAD, rather than on the underlying mechanisms. Therefore, it could be hypothesized that addressing the underlying predominant pain mechanism in the individual patient is warranted. This was the rationale for performing additional data analyses with the aim to identify subgroups based on the cut-off score of the CSI, in a patient group including both women with chronic INP and chronic WAD, distinguishing a group with and without distinct self-reported CS symptoms. Subsequently, these subgroups were compared with respect to pain, pain-related disability, health-related quality of life, cognitive deficits, experimental CS measures, and compared to healthy controls. Furthermore, associations among these variables were explored. Two subgroups based on the CSI cut-off score (40 of 100) were defined within the group of chronic INP and chronic WAD patients. The most important results of these additional data analyses are shown in **tables 2** and **3** but for detailed tables and figures we refer to the appendix.

Table 2 Summary of the results of the additional data analyses regarding pain, pain-related disability, quality of life, cognitive deficits, hyperalgesia, and efficacy of CPM in the subgroup reporting symptoms indicative of CS (n= 38) compared to the subgroup without indication of CS (n= 24), and healthy pain-free controls.

Deficits present in the subgroup with a CSI ≥ 40/100 compared to healthy women	**Deficits present in the subgroup with a CSI ≥ 40/100** compared to the subgroup with a CSI < 40/100	**Deficits present in the subgroup with a CSI < 40/100** compared to healthy women
Neck pain	Higher neck pain intensity	Neck pain
Pain-related disability & decreased quality of life	Higher pain-related disability & decreased quality of life	Pain-related disability & decreased quality of life
Subjective & objective cognitive deficits	Subjective & objective cognitive deficits	No differences in subjective or objective cognitive performance
Local & distant hyperalgesia	Local & distant hyperalgesia	No differences in local or distant hyperalgesia
Decreased CPM efficacy	No differences in CPM efficacy	No differences in CPM efficacy

Note: n= 28 for healthy controls, n= 18 chronic INP and 6 chronic WAD for CSI < 40/100, n= 15 chronic INP and 23 chronic WAD for CSI ≥ 40/100. The Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test with significance level α <0.017 (0.05/3)) was applied.
Abbreviations: CSI= central sensitization inventory, CPM= conditioned pain modulation.

The subgroup reporting CS symptoms indicative of CS (n= 38) displayed higher pain intensity, a higher number of painful days per week, worse pain-related disability, more limitations on health-related quality of life, more severe cognitive deficits, and increased local hyperalgesia compared to the subgroup reporting symptoms not indicative of CS (n= 24). Compared to healthy pain-free controls, only the subgroup showing distinct CS symptoms demonstrated cognitive deficits, distant hyperalgesia, and decreased CPM efficacy. In addition, moderate correlations were revealed among higher pain intensity, local and distant hyperalgesia, and worse self-reported and objective cognitive performance only in the subgroup presenting considerable CS symptoms. This study yields additional valuable insights and provides evidence for **subgroups based on the CSI** within women with **chronic INP and chronic WAD**, revealing both heterogeneity and overlap in clinical presentation in both conditions, and highlighting the need to **address** the **underlying predominant pain mechanism** displayed in the individual patient. Moreover, only in the subgroup with self-reported indications of CS worse cognitive performance was associated with experimental signs of CS. The latter association is interesting because it highlights the interwoven relationship between cognitive problems and the possible presence of predominant CS pain.

Table 3 Spearman correlations between cognitive deficits and disability characteristics, and pain intensity and experimental CS measures in chronic INP and chronic WAD patients distinguished based on the CSI cut-off.

chronic INP and chronic WAD	**Local hyperalgesia**	**Distant hyperalgesia**	**Decreased CPM efficacy**	**Higher pain intensity**
CSI ≥ 40/100 (n= 38)				
Self-reported cognitive deficits	~	~		~
Objective cognitive deficits		~		
Disability characteristics				~
CSI < 40/100 (n= 24)				
Self-reported cognitive deficits				
Objective cognitive deficits				
Disability characteristics				

Note: ~ =measurements are correlated in the same direction. All measurements are presented in the impaired form. To correct for multiple comparisons, correlations significant at a statistical threshold level of $p< 0.01$ (2-tailed) were deemed significant. Blanco cell = non-significant correlations. n= 67 chronic neck pain patients (35 chronic INP, 32 chronic WAD) (5 missings for the CSI). n= 18 chronic INP and 6 chronic WAD for CSI score of < 40/100, n= 15 chronic INP and 23 chronic WAD for CSI score of ≥ 40/100.
Abbreviations: CPM= conditioned pain modulation, CSI= central sensitization inventory.

Part III: The relationship between cognitive performance, cognitive stress, and central sensitization in patients with chronic whiplash associated disorders and fibromyalgia

The **third** and last **overall objective** of this thesis was addressed in **part III**, which consists of a randomized crossover study exploring interactions between cognitive performance, cognitive stress, and CS in patients with chronic WAD and FM. Besides the growing evidence for cognitive deficits, CS, and reduced quality of life in patients with chronic WAD and FM, studies examining relationships among these features in both disorders were limited. Accordingly, we performed baseline case-control comparisons in the larger crossover study, described in *chapter 6*. Furthermore, limited research was present concerning the influence of cognitive stress and relaxation on central pain modulation in patients with chronic WAD and FM. Therefore, the results of the intervention part of the randomized crossover study, outlined in *chapter 7,* attempt to fill this research gap.

Significant features of CS were demonstrated in both patients with chronic WAD and FM compared to healthy controls [32]. Similar to the results of previous studies [49, 50], enhanced temporal summation of pain or increased bottom-up sensitization was

observed in patients with chronic WAD and FM compared to healthy controls. Furthermore, deep-tissue hyperalgesia was revealed in patients with FM compared to the other study groups. Remarkably, efficacy of CPM was comparable between all study groups, which is in contrast with our results of decreased CPM efficacy in patients with chronic WAD reported in part II. Maybe these incongruent results are due to the different CPM paradigms that were applied in both studies, or due to the fact that only women were included in part II, whereas the study enclosed in part III also enrolled men.

With respect to objective **cognitive performance**, we demonstrated reduced selective and sustained attention in patients with FM compared to healthy controls [32]. Furthermore, we revealed reduced sustained attention in the chronic WAD group. Based on increased Stroop interference effects demonstrated only in FM patients, it seems that chronic WAD patients are capable to inhibit irrelevant information, whereas FM patients have problems with this attending ability indicating dysfunctional cognitive inhibition. Additionally, FM patients experienced problems to inhibit distraction stimuli. In line with accumulating evidence, patients with FM also demonstrated reduced working memory capacity compared to controls [51]. Interestingly, the results of the Operation Span Task (OSPAN) showed normal working memory capacity in chronic WAD patients, however, the results of the TMT part B applied in part II showed decreased working memory capacity in patients with chronic WAD.

Furthermore, distinct limitations on **health-related quality of life** were demonstrated in patients with chronic WAD and FM compared to controls [32]. These results are in accordance with the observed limitations on health-related quality of life in women with chronic WAD examined in *chapter 3* [31]. Worse scores in the FM group on domains of physical health were detected in comparison with chronic WAD patients.

Based on the above summarized findings, we hypothesized that both overlapping and different underlying mechanisms exist between patients with chronic INP, chronic WAD and FM. Nevertheless, research investigating pathophysiological differences between these conditions remained scarce. Therefore, it seemed valuable to perform an **additional case-control study**, including the **three chronic musculoskeletal pain conditions** examined in the present thesis. The results of this additional study are added to this dissertation as appendix and will be discussed in this section. In particular, we aimed to further explore differences in underlying mechanisms and their relationships focusing on cognitive deficits, self-reported symptoms of CS, widespread hyperalgesia, and efficacy of CPM in women with chronic INP, chronic WAD and FM compared to healthy women. Secondly, we aimed to explore associations between cognitive deficits and measures of CS in all study groups.

Self-reported cognitive deficits were higher in all patient groups compared to healthy controls (detailed figures of the results are presented in appendix). Patients with FM reported more severe cognitive deficits compared to patients with chronic INP. Worse

objective cognitive performance was revealed in patients with chronic INP (psychomotor vigilance task (PVT) lapses), chronic WAD (Stroop, PVT) and FM (Stroop, PVT, OSPAN) compared to controls. In contrast with the results of the Stroop task found in *chapter 6*, we did find in this study longer Stroop reaction times in women with chronic WAD compared to healthy women. Based on these results, we can infer that patients with chronic WAD also encounter problems with selective attention. Also, the study described in *chapter 6* and this study have demonstrated longer Stroop reaction times in patients with FM compared to controls. The latter may indicate a general slowing down of information processing in patients with FM. Regarding the OSPAN, chronic WAD patients showed normal working memory capacity in both studies. In contrast, FM patients presented reduced working memory capacity compared to healthy controls in each study.

Furthermore, only chronic WAD and FM patients reported average CSI scores higher than 40 of 100, indicating significant self-perceived signs of CS, with FM patients reporting more extensive CS symptoms compared to patients with chronic WAD. Widespread pressure hyperalgesia was demonstrated in patients with chronic WAD and FM, but not in chronic INP patients. Remarkably, **CPM efficacy** was again **comparable** between all groups, which is in line with the results of *chapter 6* but in contrast with the results of part II. In addition, as summarized in **table 4**, moderate to strong correlations were revealed among more severe subjective and objective cognitive deficits, and more self-perceived and experimental signs of CS in all patient groups.

In summary, this thesis revealed important differences between women with chronic INP, chronic WAD, and FM compared to healthy women, providing evidence for **CS associated with cognitive deficits** only in patients with **chronic WAD** and **FM**. Similar to the findings of part II, CS could not be demonstrated at a group level in chronic INP patients, while cognitive deficits were present, however, to a lesser degree compared with chronic WAD and FM patients. Furthermore, more indices of CS, more severe cognitive deficits, and more limitations on health-related quality of life were found in patients with FM compared to patients with chronic WAD. Accordingly, the chronic INP group may be situated somewhere at the beginning of the continuum of chronic musculoskeletal pain, followed by the chronic WAD group, and the FM group may be the far end of this continuum. As such, the more the experienced pain is centrally driven with increasing severity and complexity of associated symptoms, the more a patient is falling at the end of the chronic musculoskeletal pain continuum.

As can be seen in **table 5**, regarding the **associations** found in *chapter 6* between **cognitive performance** and **CS** in the chronic WAD group, only deep-tissue hyperalgesia was significantly correlated with cognitive performance. Possible explanations for the scarcely observed relationships between cognitive performance and CS in the participants with chronic WAD were obscure and needed further research, which we performed in the study enclosed in *chapter 3* and in the above outlined additional study. According to our

Table 4 Summary of the spearman correlations between self-reported and objective cognitive deficits, pressure hyperalgesia, decreased CPM efficacy, and self-reported CS symptoms (CSI) in patients with CINP, CWAD and FM, and healthy pain-free controls demonstrated in the additional case-control study.

	Subjective cognitive deficits (mPDQ)	**Impaired selective attention** (Stroop)	**Cognitive inhibition** (Stroop Interference)	**Impaired sustained attention** (PVT)	**Impaired working memory** (OSPAN)
Chronic INP (n= 17)					
Widespread hyperalgesia					
Decreased CPM efficacy			~		
Higher self-reported CS symptoms	~	~	~	~	
Chronic WAD (n= 11)					
Widespread hyperalgesia	~	~	~	~	~
Decreased CPM efficacy					
Higher self-reported CS symptoms	~	~		~	
FM (n= 29)					
Widespread hyperalgesia	~			~	
Decreased CPM efficacy					
Higher self-reported CS symptoms	~	~		~	

Note: ~ =measurements are correlated in the same direction. All measurements are presented in the impaired form. Spearman correlations were significant at the 0.05 level or below the 0.01 level or below the 0.001 level (2-tailed). Blanco cell = non-significant correlations.
Abbreviations: mPDQ= modified Perceived Deficits Questionnaire, PVT= psychomotor vigilance task, OSPAN= operation span task, CPM= conditioned pain modulation, CS= central sensitization.

hypothesis, we could reveal numerous associations between decreased cognitive performance and signs of CS in women with chronic WAD in *chapter 3* and in the additional study. In addition, reduced health-related quality of life was associated with worse cognitive performance in chronic WAD and partly in FM patients.

Table 5 Direction of the correlations between cognitive impairment, and impaired central pain modulation, and self-reported health-related quality of life observed in *chapter 6* in patients with chronic WAD and FM.

	Impaired selective attention (Stroop)		**Impaired sustained attention** (PVT)		**Impaired working memory** (OSPAN)	
	chronic WAD	**FM**	**chronic WAD**	**FM**	**chronic WAD**	**FM**
Deep-tissue hyperalgesia	1/~			~		
Increased TS of pain		1/~				1/~
Decreased CPM efficacy				~		
Reduced QoL (SF-36)	~	1/~	~	~		

Note: 1/~: measurements are oppositely correlated, ~: measurements are correlated in the same direction. All measurements are presented in the impaired form. Abbreviations: QoL= Quality of Life, SF-36= 36-item Short-Form Health Survey Questionnaire, PVT= psychomotor vigilance task, OSPAN= operation span task, TS= temporal summation, CPM= conditioned pain modulation.

Based on the results of the intervention part of the randomized crossover study described in *chapter 7* both acute cognitive stress and a single relaxation session enhanced temporal summation of pain in patients with FM, denoting increased nociceptive transmission to the brain [52]. Therefore, it can be assumed that a single progressive muscle relaxation session as well as acute cognitive stress may have negative acute effects on pain modulation in patients with FM, while acute cognitive stress and relaxation reduced temporal summation of pain in patients with chronic WAD and healthy controls. Lower temporal summation values point towards reduced bottom-up sensitization, possibly due to a change in brain focus as a result of distraction. In contrast, endogenous pain inhibition worsened in response to both relaxation and cognitive stress in healthy people, and in patients with chronic WAD and FM. Possibly, performing the cognitive tasks served as a high **cognitive stressor** for patients with FM, encountering attention and memory problems [53, 54], which further burdened the central nervous system (CNS) leading to further disinhibition and more pain. Noteworthy, the findings of *chapter 6* and the additional study showed that patients with chronic WAD demonstrated less attention and memory problems compared to FM patients [55]. Therefore, the chronic WAD patients may have experienced the cognitive tests as less challenging and hence less stressful. Accordingly, this could have led to diminished temporal summation of pain. On the

contrary, it is possible that the cognitive tasks diminished temporal summation of pain as a result of the "distraction effect" in patients with chronic WAD [56]. Regarding the **progressive muscle relaxation** therapy, the format of the relaxation may have served as a physical stressor in patients with FM, but not in patients with chronic WAD and controls. It may be that some FM patients tensed their (already painful) muscles too tightly and focused their attention even more on the pain during the relaxation session, which could have resulted in exacerbating pain accompanied with enhanced temporal summation of pain.

Clinical implications

Based on the results of the first systematic review, we can infer that structural and functional brain alterations are closely related to clinical features of pain [57]. It became clear that chronic pain is associated with a complex interplay among various brain regions and networks accompanied with alterations in areas that are often engaged in emotional, motivational, and cognitive processing of pain. Therefore, it can be recommended that the rehabilitation of patients with chronic musculoskeletal pain has to be **biopsychosocially**-driven and that the **CNS** including the brain has to be addressed. Hence, therapy approaches should tackle the observed affective-, motivational-, cognitive-, and psychosocial dysfunctions in the individual patient.

The findings of this dissertation indicated that women with chronic INP, chronic WAD and FM are characterized by similar but also prominently different underlying mechanisms. The results can be interpreted in the light of the conceptual framework of the continuum of chronic musculoskeletal pain as outlined in the introduction and previously discussed. It can be proposed that FM patients are falling at the end of the continuum because the medical diagnosis of FM most often implies the presence of predominant CS pain [58, 59], while chronic WAD is associated but not uniformly characterized by CS [60], and CS is not a characteristic feature in chronic INP.

But it should be mentioned that these similarities and differences are evaluated on a group level and may differ substantially at the individual patient level, regardless of the diagnosis. Based on these results, it can be advocated that **disability**, **cognitive deficits**, signs of **CS**, and their possible **interrelationships** should be **evaluated** and **treated** with the aim of providing more effective and individually focused therapy for patients with chronic INP, chronic WAD and FM. Questionnaires such as the Neck Disability Index or the Pain Disability Index, the Short Form 36-item Health survey, and the modified Perceived Deficits Questionnaire are recommended to evaluate and re-evaluate disability, health-related quality of life, and cognitive deficits in patients with chronic INP, chronic WAD and FM. Furthermore, the TMT could be used as a quickly administered test to examine cognitive performance. Additionally, the importance of assessing the **underlying predominant pain mechanism** in the individual patient is crucial. Therapists should not only be aware of the possibility of CS in patients with chronic WAD and FM but also in patients with chronic INP because, at the individual patient level, it is still possible that CS is present in patients with chronic INP. Therefore, therapists should be able to **recognize CS**. Clinical guidelines for recognizing and treating CS are available in current literature [60, 61]. The evaluation of self-reported CS symptoms for example with the CSI (by interpreting the cut-off score of 40 of 100) combined with clinical examination (e.g. assessment of pressure pain thresholds or cold sensitivity at sites distant from the symptomatic region), and evaluation of the treatment response, all together can contribute to the recognition

of CS [60]. Subsequently, the therapeutic approach should be personalized for each patient and clinicians should account for CS when designing the treatment plan in case of CS [62], regardless of diagnosis. The need to address the underlying predominant pain mechanism is confirmed by the results of the additional data analyses identifying subgroups based on the CSI in women with chronic WAD and chronic INP. The fact that this subgroup also comprised 15 chronic INP patients affirms our hypothesis that at the individual patient level, CS can be present as underlying pain mechanism in patients with chronic INP.

When the clinical picture of pain patients is characterized or predominated by CS or when maladaptive illness perceptions are present [63], **pain neuroscience education** should be applied. The goals of such education are to reconceptualize pain and to change inappropriate pain beliefs; reduce maladaptive attitudes and cognitions in relation to pain; and subsequent increase participation in active treatment [64, 65]. Previous research demonstrated significant decreases in pain-related disability and hyperalgesia after pain neurophysiology education in chronic WAD patients, and revealed improved health status and endogenous pain inhibition in patients with FM [65, 66]. In addition, a recent study of our research group unpublished results observed in patients with chronic spinal pain including chronic INP and chronic WAD patients the effectiveness of a time-contingent biopsychosocial approach combining pain neuroscience education with cognition-targeted exercise therapy for reducing symptoms of CS and disability, and improving pain cognitions, and health-related quality of life. On the basis of our results, also cognitive deficits, such as attention, concentration, and memory problems, should be taken into account in the application of pain education in these patients. In addition, **cognitive behavioral therapy** can be recommended and is able to decrease pain-related disability and post-traumatic stress, and improve quality of life in patients with chronic WAD [67, 68] as well as reduce pain catastrophizing in patients with FM [69]. In acute and subacute chronic pain patients, early pain education and cognitive behavioral therapy are important for preventing the transition to chronicity [70]. When applying hands-on therapies in patients with predominant CS pain, clinicians should remember that therapeutic interventions triggering more pain will serve as a new peripheral source of nociceptive input and thus will sustain the CS process [71].

The brain MRI results furthermore indicated that persistent pain in chronic WAD patients should be interpreted, at least in part, as a result of structural plasticity of the CNS associated with alterations in GM morphology and WM microstructure of regions involved in pain and cognitive processing. As such, clinicians should be aware of the observed subtle structural brain alterations in chronic WAD patients, and the associations with cognitive deficits, pain catastrophizing and hypervigilance, and CS symptoms. This again highlights that therapy approaches for chronic WAD should address the **brain** and take into account **neuroplasticity of the CNS,** like e.g. cognitive behavioral therapy which has

been demonstrated to reverse regional GM volume decreases associated with reduced pain catastrophizing and decreased cognitive deficits in other chronic CS pain patients [72, 73]. Multiple studies have indeed shown in other chronic pain conditions that decrease in GM morphology is at least partially reversible when underlying pain is adequately treated [14, 74, 75].

In chronic INP patients, only cognitive deficits were related to decreased regional GM morphology, and GM morphological alterations or CS could not be revealed. Accordingly, fewer indications are currently available for a role of brain alterations and CNS neuroplasticity in the pathophysiology of chronic INP at a group level.

Finally, based on the results of the study in patients with chronic WAD and FM investigating the effects of cognitive stress on endogenous pain modulation [52], therapists should be aware of the possible negative influences of stressors such as cognitive demanding tasks on endogenous pain modulation, and thus should assess the patient's individual ability to cope with stress. This fits in the clinical biopsychosocial assessment approach which we recommend for patients with chronic pain [76]. This assessment aims to establish the underlying predominant pain mechanism as well as the provoking and sustaining biopsychosocial factors in the individual patient. In this type of assessment, the influence of different factors associated with chronic pain such as emotional factors (e.g. chronic stress, pain-related fear), cognitive factors (e.g. catastrophizing), behavioral factors (e.g. avoidance or persistence behavior), social factors (e.g. relationship with partner, work), and motivation (e.g. readiness to change) should be determined. This extensive biopsychosocial assessment is an important first step in adequate patient-centered pain neuroscience education and successful treatment of patients with chronic pain.

Taken together, we suggest an **individually-tailored** multicomponent **biopsychosocial** assessment and rehabilitation in which the underlying pathophysiological mechanisms and their interrelationships should be taken into account.

Limitations and Strengths

The study limitations of each study enclosed in this dissertation have been discussed in detail in the different manuscripts. In the following section, the most important **limitations** that must be taken into account when interpreting the results, will be discussed.

To correctly interpret the results of our systematic reviews, it is important to mention that different MRI acquisition and analyzing techniques were used in the included studies which can affect the study results. Furthermore, the various brain imaging techniques used in *chapter 2* hampers the possibility of comparison. Next, the included studies administered different scales or questionnaires to measure clinical features of pain. This might give difficulties comparing results of different studies. In addition, various studies included in the second systematic review did suffer from certain risks of bias, which could affect their results and implies the possibility to infer only cautious conclusions.

For the original studies, it should be mentioned that the cross-sectional nature implies that no conclusions about the causality of the observed correlations can be drawn. Furthermore, because the case-control studies included in part II only examined women, caution should be taken when generalizing the results to the total chronic INP and chronic WAD population. Accordingly, the external validity of the results described in *chapters 3 to 5* is limited to adult female patients with chronic INP and chronic WAD, in particular chronic WAD patients diagnosed as WAD II A, B, or C. Nevertheless, we are convinced that this limitation is a strength as well because this way we avoided bias in our results due to differences between men and women concerning pain sensitivity, pain processing, and brain structure [77-79], and because this way there was less heterogeneity in our study sample. Noteworthy, the prevalence of both traumatic [80] and idiopathic [81] chronic neck pain is consistently higher in women. Also, with regard to interpretation of the cognitive test results, the possible presence of malingering or feigned cognitive deficits in women with chronic INP and, in particular, in women with chronic WAD must be considered. This limitation was avoided in the additional study in which patients with chronic INP, chronic WAD, and FM performed, besides the cognitive tests, the Rey 15-item memory test to exclude the presence of malingering. Furthermore, the focus of this dissertation was not on psychological correlates of pain such as posttraumatic stress, anxiety, depression, pain catastrophizing and hypervigilance, although these important features were also assessed and could influence the observed differences and interrelationships. However, these psychological aspects will be included in our further data analyses in patients with chronic INP, chronic WAD and FM.

Metrics derived from diffusion-weighted imaging data using the tensor model are indirect measures that relate to but do not directly quantify WM microstructural features and are influenced by methodological and biological factors [82]. These metrics cannot disentangle the individual microscopic contributions at the voxel level and therefore should be interpreted with caution. It remains unclear whether decreased fractional anisotropy is due to changes in membrane permeability, organelles, axon thickness, fiber density, or degree of myelination [82]. It has been suggested that alterations in radial diffusivity combined with unchanged axial diffusivity reflect demyelination [45], but the interpretation of these metrics has been a topic of controversy [83]. Another limitation of DTI analyses of single-shell data pertains to the inability of the tensor model to adequately characterize diffusion in voxels containing complex fiber architecture, such as crossing fibers [82]. In the brain however voxels often contain fiber populations with more than one dominant orientation.

Nevertheless, also several **strengths** of this dissertation can be outlined. First, the methods used for screening and scoring the articles included in our systematic reviews were completed by two independent blinded reviewers. Second, the Newcastle Ottawa Scale used to assess risk of bias was modified by adding two items specifically developed for the topic of both systematic reviews. Consequently, the methodological quality and risk of bias of the brain imaging studies could have been evaluated more thoroughly giving a more accurate view on the pre-processing of the brain imaging analyses, and on the brain imaging acquisition and quality control.

Third, the case-control studies enclosed in *chapters 3* to *7* are the first to address the outlined clinically relevant research questions regarding differences in underlying mechanisms between patients with chronic INP, chronic WAD and FM. This research has increased our knowledge of these musculoskeletal pain conditions substantially, which could steer further research and development of more effective individualized therapy focused on the underlying pathophysiological mechanisms.

The large sample size of the case-control studies described in part II is a considerable strength as many research studies in the domain of chronic pain and in particular of brain imaging are limited by small sample sizes, and thus potentially lack sufficient statistical power.

Fourth, an important strength of the case-control studies was the comparability of the groups in age, sex, body mass index, and education level. Neck pain duration and frequency of neck pain complaints were comparable for the chronic neck pain groups as well. Another important strength of all original research studies was that we anticipated sources of bias, such as use of medications, caffeine, alcohol, and nicotine; menstrual phase; pregnancy; and performance of heavy physical exertion on the assessment day.

Finally, the brain MRI studies addressing relationships between alterations in GM morphology and WM microstructure, and self-reported and experimental features of cognition, pain, and CS in women with chronic INP and chronic WAD compared to healthy women are highly innovative.

Directions for further research

This dissertation has increased our knowledge concerning the underlying mechanisms and their relationships in patients with chronic INP, chronic WAD and FM as well as their differences. Based on our increased understanding, interesting new research questions arose and some questions remained unresolved. Accordingly, important recommendations for further research can be proposed.

As mentioned before, no conclusions about the causality of the observed relationships can be drawn based on the results of the cross-sectional studies. Hence, further **longitudinal research** is warranted to investigate if CS, pain-related disability, and reduced health-related quality of life lead to cognitive deficits or vice versa. Furthermore, research should investigate which therapy strategies are able to reduce and/or prevent CS and associated pain-related disability and psychosocial aspects in patients with chronic musculoskeletal pain.

In addition, longitudinal studies, not only in patients with chronic pain but also in patients with acute and subacute pain, are highly warranted to unravel the **cause-effect relationships** between brain alterations, and chronic pain to answer the question if these alterations lead to chronic pain or vice versa, or an interaction between both. Longitudinal brain imaging research needs to explore the exact **temporal characteristics** of structural and functional brain alterations. As such, this research could increase our insight in which brain alterations are mediating the transition from acute to chronic pain. To date, this transition is hypothesized to comprise of four phases: predisposition, injury or inciting event (e.g. whiplash), a transition period, and a maintenance phase [1, 84]. Subsequently, research has to investigate which therapies can intervene in which stage of this transition for preventing pain chronification.

Also, much more effort is needed to explore which **therapies** are able to **reverse** the observed structural **brain alterations** in patients with chronic WAD, and if normalizing of these brain alterations is associated with diminishment of pain and related disability, less signs of CS, decreased pain catastrophizing and hypervigilance, and less severe cognitive deficits. To this end, longitudinal research is warranted to unravel which therapeutic approaches can re-shape the brain and decrease the associated burden in patients with chronic pain.

Our findings have yielded innovative evidence for structural brain alterations in women with chronic WAD compared to women with chronic INP and healthy women. However, we are convinced that the present thesis has investigated only one piece of the puzzle regarding possible brain alterations in patients with trauma-induced and non-traumatic chronic neck pain. Therefore, we want to emphasize the need for further research using **functional brain imaging** techniques such as (resting-state) functional MRI or Electro-

encephalography in order to disentangle possible functional brain alterations in both chronic neck pain populations. Also, whole brain research focusing on structural and functional network and connectivity analyses in these patients compared to healthy pain-free controls can be recommended. Furthermore, the exact underlying neurobiological mechanisms responsible for decreased regional GM morphology in chronic WAD patients remain unclear and have to be elucidated with further studies applying sophisticated and sensitive brain imaging techniques.

Moreover, the **relationships** between cognitive performance and CS measures, and both structural and functional brain characteristics should be further explored to gain more insight in the underpinning mechanisms of chronic pain, and to uncover the specific role of brain alterations in the persistent complaints of chronic pain patients. For example, it could be interesting to investigate associations between temporal summation of second pain or objective quantitative sensory testing measures of CS (e.g. the nociceptive flexion reflex (NFR) threshold), and brain alterations in patients with chronic WAD and FM. The latter could add valuable insights into the structural and functional brain correlates of CS. Furthermore, associations between brain parameters and objective cognitive deficits for example examined with performance-based cognitive tests (OSPAN, STROOP, PVT) are valuable to investigate. Also, exploration of relationships between experimental measures of CS and cognition on the one hand, and pain catastrophizing, hypervigilance but also pain-related fear, anxiety, stress, and illness perceptions should be further investigated in patients with chronic INP and chronic WAD in order to further unravel the biopsychosocial aspects of chronic neck pain. In addition, the influence of psychosocial correlates such as pain-related fear and post-traumatic stress on structural and functional brain characteristics in patients with chronic WAD is an interesting avenue for further research.

Regarding our findings of abnormalities in WM microstructure in the chronic WAD group, and associations with dysfunctional CPM, it seems valuable to further explore other alterations in WM in patients with chronic WAD but likewise in acute and subacute whiplash patients. WM of the brain also consists of glial cells and research has found that glial activation plays an important role in the induction and maintenance of chronic pain [85]. Moreover, preliminary evidence is present for effects of minor traumatic brain injuries on glial cells, such as proliferation and microglial activation [86]. Hence, research into possible alterations in brain **glial activation** in WAD is of high interest for example with integrated Positron Emission Tomography-MRI [85]. This research avenue would also be valuable because evidence suggesting that activation of glia cells and neuro-glial interactions are mediators of sensitization has been emerged [87, 88]. Also, advanced models based on the high angular resolution DWI acquisition strategy to provide more robust estimates of the fiber orientation in WM are recommended for further research in patients with chronic INP, chronic WAD, and FM.

We recommend for future research that the assessment of CS in patients with chronic pain should include besides the tests applied in this thesis, also more **objective experimental tests** such as the evaluation of the NFR threshold.

To date, there is still a lack of absolute diagnostic criteria for identifying the presence of CS in pain patients [89]. Our research group has proposed a valuable **clinical algorithm** for the **recognition of CS** in chronic pain patients in clinical practice [60, 61]. However, this algorithm should be validated in future studies. Also, it should be further discovered how researchers and clinicians can examine the presence of dysfunctional facilitating or inhibiting pain modulatory mechanisms at the individual level. Possibly, different therapy strategies are necessary to restore the balance of endogenous pain modulation in case of more prominent dysfunctional facilitating or inhibiting pain modulation.

To conclude, it remains an important challenge for researchers and therapists to develop effective therapy strategies for patients with chronic INP, chronic WAD and FM but our results pave the way for the development of novel and more effective treatment approaches for these pain conditions.

General conclusion

We can conclude that moderate evidence exists for relationships between clinical pain measures, and structural and functional brain alterations within regions or networks involved in somatosensory, affective-motivational, and cognitive pain processing in patients with chronic musculoskeletal pain. Furthermore, this thesis revealed important differences between patients with chronic INP, chronic WAD and FM, providing evidence for CS associated with subjective and objective cognitive deficits only in patients with chronic WAD and FM. Additionally, this research has demonstrated disability associated with cognitive deficits in patients with chronic WAD and FM, and to a significantly lesser extent in chronic INP patients. Higher disability, more severe cognitive deficits, and more indices of CS were revealed in patients with FM compared to chronic WAD and chronic INP patients. Accordingly, the chronic INP group may be situated somewhere at the beginning of the continuum of chronic musculoskeletal pain, followed by the chronic WAD group, and the FM group may be the far end of this continuum. As such, the more the experienced pain is centrally driven, the more a patient is falling at the end of this continuum.

When comparing patients with chronic INP and chronic WAD, distant hyperalgesia and decreased CPM efficacy were demonstrated in chronic WAD but not in chronic INP patients, indicating the presence of CS, at a group level, only in patients with chronic WAD. Nevertheless, when subgrouping based on self-reported symptoms of CS, instead of on diagnosis, only the subgroup showing distinct CS symptoms (also comprising women with chronic INP) demonstrated cognitive deficits, distant hyperalgesia, and decreased CPM efficacy compared to healthy controls. In addition, only in this subgroup with considerable self-perceived CS symptoms associations between more severe cognitive deficits and distant hyperalgesia were found. These findings highlight the need to address the underlying predominant pain mechanism displayed in the individual patient.

The present thesis has furthermore demonstrated abnormalities in WM microstructure and decreased GM morphology in regions associated with pain and cognitive processing without detecting brain microhemorrhages related to trauma in women with chronic WAD compared to women with chronic INP and healthy women. In patients with chronic WAD, decreased regional GM volume was associated with more severe cognitive deficits and more signs of CS. In addition, novel evidence is provided for associations between dysfunctional CPM and the degree of regional WM deficits in chronic WAD. This yields innovative evidence for underlying WM microstructural correlates of disturbed endogenous pain inhibition. Accordingly, these results emphasize the role of structural brain alterations in patients with traumatic chronic neck pain (WAD) compared to patients with non-traumatic chronic INP. Together, these findings indicate a possible negative mediating role of the trauma in patients with chronic WAD.

We recommend that disability, cognitive deficits, and CS, and their relationships should be evaluated and treated in patients with chronic INP, chronic WAD and FM with the aim of providing more effective and individually focused therapy, which tackles the observed deficits. Based on the results of this thesis, it can be recommended that therapy approaches for chronic WAD and FM should take into account the role of CNS neuroplasticity. Further research is warranted to investigate which therapies can decrease CS, cognitive deficits, and disability in patients with chronic INP, chronic WAD and FM. Furthermore, longitudinal research should examine which therapeutic approaches can reverse the observed structural brain alterations, and should assess if normalization of these brain alterations is associated with less severe clinical dysfunctions.

In conclusion, the results of this dissertation have unraveled important pieces of the complex pathophysiological puzzle underlying chronic INP, chronic WAD and FM, and have uncovered both differences and overlapping similarities between these chronic musculoskeletal pain conditions. The novel findings increase our understanding of these conditions substantially, and could steer further research and contribute to more effective individually tailored therapy approaches.

General Discussion

References

1. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 2015; 87:474-491.
2. Martinez-Jauand M, Sitges C, Rodriguez V, Picornell A, Ramon M, Buskila D, Montoya P. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. *Eur J Pain* 2013; 17:16-27.
3. Tsang A, Von Korff M, Lee S, Alonso J, Karam E, Angermeyer MC, Borges GL, Bromet EJ, Demytteneare K, de Girolamo G, de Graaf R, Gureje O, Lepine JP, Haro JM, Levinson D, Oakley Browne MA, Posada-Villa J, Seedat S, Watanabe M. Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. *J Pain* 2008; 9:883-891.
4. Blankstein U, Chen J, Diamant NE, Davis KD. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology* 2010; 138:1783-1789.
5. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron* 2008; 60:570-581.
6. Loggia ML, Berna C, Kim J, Cahalan CM, Martel MO, Gollub RL, Wasan AD, Napadow V, Edwards RR. The lateral prefrontal cortex mediates the hyperalgesic effects of negative cognitions in chronic pain patients. *J Pain* 2015; 16:692-699.
7. Younger JW, Chu LF, D'Arcy NT, Trott KE, Jastrzab LE, Mackey SC. Prescription opioid analgesics rapidly change the human brain. *Pain* 2011; 152:1803-1810.
8. Ceko M, Bushnell MC, Fitzcharles MA, Schweinhardt P. Fibromyalgia interacts with age to change the brain. *Neuroimage Clin* 2013; 3:249-260.
9. Henderson LA, Gandevia SC, Macefield VG. Gender differences in brain activity evoked by muscle and cutaneous pain: a retrospective study of single-trial fMRI data. *Neuroimage* 2008; 39:1867-1876.
10. Mogil JS, Bailey AL. Sex and gender differences in pain and analgesia. *Prog Brain Res* 2010; 186:141-157.
11. Matsuzawa-Yanagida K, Narita M, Nakajima M, Kuzumaki N, Niikura K, Nozaki H, Takagi T, Tamai E, Hareyama N, Terada M, Yamazaki M, Suzuki T. Usefulness of antidepressants for improving the neuropathic pain-like state and pain-induced anxiety through actions at different brain sites. *Neuropsychopharmacology* 2008; 33:1952-1965.
12. Borchgrevink G, Smevik O, Haave I, Haraldseth O, Nordby A, Lereim I. MRI of cerebrum and cervical columna within two days after whiplash neck sprain injury. *Injury* 1997; 28:331-335.
13. Karlsborg M, Smed A, Jespersen HF, Stephensen SL, Cortsen ME, Jennum PJ, Herning GM, Korfitsen E, Werdelin LM. [Whiplash injury syndrome. A prospective study of 39 patients with whiplash injury]. *Ugeskr Laeger* 1998; 160:6211-6215.
14. Obermann M, Nebel K, Schumann C, Holle D, Gizewski ER, Maschke M, Goadsby PJ, Diener HC, Katsarava Z. Gray matter changes related to chronic posttraumatic headache. *Neurology* 2009; 73:978-983.
15. Davis KD, Moayedi M. Central Mechanisms of Pain Revealed Through Functional and Structural MRI. *Journal of Neuroimmune Pharmacology* 2013; 8:518-534.
16. Baliki MN, Apkarian AV. Perspective Nociception , Pain , Negative Moods. 2015.
17. Farmer MA, Vachon-presseau E. Pain : Acute and Chronic The Emotional Brain as a Predictor and Amplifier of Chronic Pain. 2015:0-8.
18. Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; 66:355-474.
19. Freitag P, Greenlee MW, Wachter K, Ettlin TM, Radue EW. fMRI response during visual motion stimulation in patients with late whiplash syndrome. *Neurorehabil Neural Repair* 2001; 15:31-37.
20. Linnman C, Appel L, Furmark T, Soderlund A, Gordh T, Langstrom B, Fredrikson M. Ventromedial prefrontal neurokinin 1 receptor availability is reduced in chronic pain. *Pain* 2010; 149:64-70.
21. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000; 288:1765-1768.
22. Seybold VS. The role of peptides in central sensitization. *Handb Exp Pharmacol* 2009:451-491.
23. Bakhtadze MA, Vernon H, Karalkin AV, Pasha SP, Tomashevskiy IO, Soave D. Cerebral perfusion in patients with chronic neck and upper back pain: preliminary observations. *J Manipulative Physiol Ther* 2012; 35:76-85.
24. Linnman C, Appel L, Soderlund A, Frans O, Engler H, Furmark T, Gordh T, Langstrom B, Fredrikson M. Chronic whiplash symptoms are related to altered regional cerebral blood flow in the resting state. *Eur J Pain* 2009; 13:65-70.
25. Sundstrom T, Guez M, Hildingsson C, Toolanen G, Nyberg L, Riklund K. Altered cerebral blood flow in chronic neck pain patients but not in whiplash patients: a 99mTc-HMPAO rCBF study. *Eur Spine J* 2006; 15:1189-1195.
26. Otte A, Mueller-Brand J, Nitzsche EU, Wachter K, Ettlin TM. Functional brain imaging in 200 patients after whiplash injury. *J Nucl Med* 1997; 38:1002.
27. Radanov BP, Bicik I, Dvorak J, Antinnes J, von Schulthess GK, Buck A. Relation between neuropsychological and neuroimaging findings in patients with late whiplash syndrome. *J Neurol Neurosurg Psychiatry* 1999; 66:485-489.
28. Lorberboym M, Gilad R, Gorin V, Sadeh M, Lampl Y. Late whiplash syndrome: correlation of brain SPECT with neuropsychological tests and P300 event-related potential. *J Trauma* 2002; 52:521-526.
29. Dickerson BC, Salat DH, Bates JF, Atiya M, Killiany RJ, Greve DN, Dale AM, Stern CE, Blacker D, Albert MS. Medial temporal lobe function and structure in mild cognitive impairment. *Annals of neurology* 2004; 56:27-35.
30. De Pauw R, Coppieters I, Meeus M, Caeyenberghs K, Danneels L, B C. Is traumatic and non-traumatic neck pain associated with brain alterations? A systematic review. *Accepted in Pain Physician* 2017.
31. Coppieters I., De Pauw R., Kregel J., Malfliet A., Goubert D., Lenoir D., Cagnie B., M. M. Differences between women with traumatic and idiopathic chronic neck pain and women without neck pain: interrelationships among disability, cognitive deficits, and central sensitization. *Physical Therapy* March 2017.
32. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive Performance Is Related to Central Sensitization and Health-related Quality of Life in Patients with Chronic Whiplash-Associated Disorders and Fibromyalgia. *Pain Physician* 2015; 18:E389-401.
33. Cagnie B, Coppieters I, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. *Semin Arthritis Rheum* 2014; 44:68-75.
34. Kregel J, Meeus M, Malfliet A, Dolphens M, Danneels L, Nijs J, Cagnie B. Structural and functional brain abnormalities in chronic low back pain: A systematic review. *Semin Arthritis Rheum* 2015.
35. Younger JW, Shen YF, Goddard G, Mackey SC. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. *Pain* 2010; 149:222-228.
36. As-Sanie S, Harris RE, Napadow V, Kim J, Neshewat G, Kairys A, Williams D, Clauw DJ, Schmidt-Wilcke T. Changes in regional gray matter volume in women with chronic pelvic pain: a voxel-based morphometry study. *Pain* 2012; 153:1006-1014.
37. Walitt B, Ceko M, Gracely JL, Gracely RH. Neuroimaging of Central Sensitivity Syndromes: Key Insights from the Scientific Literature. *Curr Rheumatol Rev* 2016; 12:55-87.
38. Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O. Mapping the structural core of human cerebral cortex. *PLoS Biol* 2008; 6:e159.
39. Fransson P, Marrelec G. The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *Neuroimage* 2008; 42:1178-1184.
40. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 2006; 129:564-583.
41. Jensen KB, Srinivasan P, Spaeth R, Tan Y, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Vitton O, Gracely R, Ingvar M, Kong J. Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis Rheum* 2013; 65:3293-3303.
42. Govindarajan KA, Narayana PA, Hasan KM, Wilde EA, Levin HS, Hunter JV, Miller ER, Patel VK, Robertson CS, McCarthy JJ. Cortical Thickness in Mild Traumatic Brain Injury. *J Neurotrauma* 2016.
43. Kong L, Herold CJ, Zollner F, Salat DH, Lasser MM, Schmid LA, Fellhauer I, Thomann PA, Essig M, Schad LR, Erickson KI, Schroder J. Comparison of grey matter volume and thickness for analysing cortical changes in chronic schizophrenia: a matter of surface area, grey/white matter intensity contrast, and curvature. *Psychiatry Res* 2015; 231:176-183.
44. Janve VA, Zu Z, Yao SY, Li K, Zhang FL, Wilson KJ, Ou X, Does MD, Subramaniam S, Gochberg DF. The radial diffusivity and magnetization transfer pool size ratio are sensitive markers for demyelination in a rat model of type III multiple sclerosis (MS) lesions. *Neuroimage* 2013; 74:298-305.
45. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage* 2002; 17:1429-1436.
46. Sun SW, Liang HF, Schmidt RE, Cross AH, Song SK. Selective vulnerability of cerebral white matter in a murine model of multiple sclerosis detected using diffusion tensor imaging. *Neurobiol Dis* 2007; 28:30-38.

47. Lieberman G, Shpaner M, Watts R, Andrews T, Filippi CG, Davis M, Naylor MR. White Matter Involvement in Chronic Musculoskeletal Pain. *Journal of Pain* 2014; 15:1110-1119.
48. Liu G, Ghimire P, Pang H, Wu G, Shi H. Improved sensitivity of 3.0 Tesla susceptibility-weighted imaging in detecting traumatic bleeds and its use in predicting outcomes in patients with mild traumatic brain injury. *Acta Radiol* 2015; 56:1256-1263.
49. Lemming D, Graven-Nielsen T, Sorensen J, Arendt-Nielsen L, Gerdle B. Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women. *J Rehabil Med* 2012; 44:648-657.
50. Staud R, Bovee CE, Robinson ME, Price DD. Cutaneous C-fiber pain abnormalities of fibromyalgia patients are specifically related to temporal summation. *Pain* 2008; 139:315-323.
51. Dick BD, Verrier MJ, Harker KT, Rashiq S. Disruption of cognitive function in fibromyalgia syndrome. *Pain* 2008; 139:610-616.
52. Coppieters I, Cagnie B, Nijs J, van Oosterwijck J, Danneels L, De Pauw R, Meeus M. Effects of Stress and Relaxation on Central Pain Modulation in Chronic Whiplash and Fibromyalgia Patients Compared to Healthy Controls. *Pain Physician* 2016; 19:119-130.
53. Tesio V, Torta DM, Colonna F, Leombruni P, Ghiggia A, Fusaro E, Geminiani GC, Torta R, Castelli L. Are fibromyalgia patients cognitively impaired? Objective and subjective neuropsychological evidence. *Arthritis Care Res (Hoboken)* 2015; 67:143-150.
54. Suhr JA. Neuropsychological impairment in fibromyalgia: relation to depression, fatigue, and pain. *J Psychosom Res* 2003; 55:321-329.
55. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M. Cognitive performance is related to central sensitization and health-related quality of life in patients with chronic whiplash-associated disorders and fibromyalgia. *Pain Physician* 2015; 18.
56. Eccleston C, Crombez G. Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychol Bull* 1999; 125:356-366.
57. Coppieters I, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. *J Pain* 2016.
58. Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clin Rheumatol* 2007; 26:465-473.
59. Nielsen LA, Henriksson KG. Pathophysiological mechanisms in chronic musculoskeletal pain (fibromyalgia): the role of central and peripheral sensitization and pain disinhibition. *Best Pract Res Clin Rheumatol* 2007; 21:465-480.
60. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: Application of pain neurophysiology in manual therapy practice. *Man Ther* 2010; 15:135-141.
61. Nijs J, Torres-Cueco R, van Wilgen CP, Girbes EL, Struyf F, Roussel N, van Oosterwijck J, Daenen L, Kuppens K, Vanwerweeen L, Hermans L, Beckwee D, Voogt L, Clark J, Moloney N, Meeus M. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. *Pain Physician* 2014; 17:447-457.
62. Nijs J, Goubert D, Ickmans K. Recognition and Treatment of Central Sensitization in Chronic Pain Patients: Not Limited to Specialized Care. *J Orthop Sports Phys Ther* 2016; 46:1024-1028.
63. Nijs J, Paul van Wilgen C, Van Oosterwijck J, van Ittersum M, Meeus M. How to explain central sensitization to patients with 'unexplained' chronic musculoskeletal pain: practice guidelines. *Man Ther* 2011; 16:413-418.
64. Nijs J, Meeus M, Cagnie B, Roussel NA, Dolphens M, Van Oosterwijck J, Danneels L. A modern neuroscience approach to chronic spinal pain: combining pain neuroscience education with cognition-targeted motor control training. *Phys Ther* 2014; 94:730-738.
65. Van Oosterwijck J, Nijs J, Meeus M, Truijen S, Craps J, Van den Keybus N, Paul L. Pain neurophysiology education improves cognitions, pain thresholds, and movement performance in people with chronic whiplash: a pilot study. *J Rehabil Res Dev* 2011; 48:43-58.
66. Van Oosterwijck J, Meeus M, Paul L, De Schryver M, Pascal A, Lambrecht L, Nijs J. Pain physiology education improves health status and endogenous pain inhibition in fibromyalgia: a double-blind randomized controlled trial. *Clin J Pain* 2013; 29:873-882.

6

7. Dunne RL, Kenardy J, Sterling M. A randomized controlled trial of cognitive-behavioral therapy for the treatment of PTSD in the context of chronic whiplash. *Clin J Pain* 2012; 28:755-765.
68. Nijs J, Van Oosterwijck J, De Hertogh W. Rehabilitation of chronic whiplash: treatment of cervical dysfunctions or chronic pain syndrome? *Clin Rheumatol* 2009; 28:243-251.
69. Lazaridou A, Kim J, Cahalan CM, Loggia ML, Franceschelli O, Berna C, Schur P, Napadow V, Edwards RR. Effects of Cognitive-behavioral Therapy (CBT) on Brain Connectivity Supporting Catastrophizing in Fibromyalgia. *Clin J Pain* 2016.
70. Andersen TE, Ravn SL, Roessler KK. Value-based cognitive-behavioural therapy for the prevention of chronic whiplash associated disorders: protocol of a randomized controlled trial. *BMC Musculoskelet Disord* 2015; 16:232.
71. Nijs J, Van Houdenhove B. From acute musculoskeletal pain to chronic widespread pain and fibromyalgia: application of pain neurophysiology in manual therapy practice. *Man Ther* 2009; 14:3-12.
72. de Lange FP, Koers A, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I. Increase in prefrontal cortical volume following cognitive behavioural therapy in patients with chronic fatigue syndrome. *Brain* 2008; 131:2172-2180.
73. Seminowicz DA, Shpaner M, Keaser ML, Krauthamer GM, Mantegna J, Dumas JA, Newhouse PA, Filippi CG, Keefe FJ, Naylor MR. Cognitive-behavioral therapy increases prefrontal cortex gray matter in patients with chronic pain. *J Pain* 2013; 14:1573-1584.
74. Gwilym SE, Filippini N, Douaud G, Carr AJ, Tracey I. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheum* 2010; 62:2930-2940.
75. Rodriguez-Raecke R, Niemeier A, Ihle K, Ruether W, May A. Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *J Neurosci* 2009; 29:13746-13750.
76. Wijma AJ, van Wilgen CP, Meeus M, Nijs J. Clinical biopsychosocial physiotherapy assessment of patients with chronic pain: The first step in pain neuroscience education. *Physiother Theory Pract* 2016; 32:368-384.
77. Ruigrok AN, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev* 2014; 39:34-50.
78. Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. *J Pain Res* 2015; 8:311-320.
79. van Hemmen J, Saris IM, Cohen-Kettenis PT, Veltman DJ, Pouwels PJ, Bakker J. Sex Differences in White Matter Microstructure in the Human Brain Predominantly Reflect Differences in Sex Hormone Exposure. *Cereb Cortex* 2016.
80. Guez M, Hildingsson C, Nilsson M, Toolanen G. The prevalence of neck pain: a population-based study from northern Sweden. *Acta Orthop Scand* 2002; 73:455-459.
81. Haldeman S, Carroll L, Cassidy JD. Findings from the bone and joint decade 2000 to 2010 task force on neck pain and its associated disorders. *J Occup Environ Med* 2010; 52:424-427.
82. K M. Curran LE, A. Leemans. Quantitative DTI measures, in Wim van Hecke LE, Stefan Sunaert (ed). Diffusion tensor imaging: a practical handbook. Springer, New York, 2016, pp 65-85.
83. Wheeler-Kingshott CA, Cercignani M. About "axial" and "radial" diffusivities. *Magn Reson Med* 2009; 61:1255-1260.
84. Apkarian AV, Baliki MN, Farmer MA. Predicting transition to chronic pain. *Current Opinion in Neurology* 2013; 26:360-367.
85. Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, Hill E, Hsu S, Izquierdo-Garcia D, Ji RR, Riley M, Wasan AD, Zurcher NR, Albrecht DS, Vangel MG, Rosen BR, Napadow V, Hooker JM. Evidence for brain glial activation in chronic pain patients. *Brain* 2015; 138:604-615.
86. Andersson HC, Anderson MF, Porritt MJ, Nodin C, Blomstrand F, Nilsson M. Trauma-induced reactive gliosis is reduced after treatment with octanol and carbenoxolone. *Neurol Res* 2011; 33:614-624.
87. Arendt-Nielsen L. Central sensitization in humans: assessment and pharmacology, in Schaible H-G (ed). Handbook of experimental pharmacology. Springer Berlin Heidelberg, 2015, pp 81.
88. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? *Pain* 2013; 154 Suppl 1:S10-28.
89. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 2011; 152:S2-15.

English Summary

"There is plenty of tunnel at the end of the light."
- Jerry Fodor

English Summary

English Summary

The core aim of this dissertation was to improve our knowledge of the mechanisms and their possible interrelationships underlying the persistent, complex, and often unexplained symptoms in patients with chronic idiopathic neck pain (INP), chronic whiplash associated disorders (WAD) and fibromyalgia (FM). The core aim was furthermore to unravel differences in the nature and severity of the underpinning mechanisms between these three heterogeneous conditions with chronic musculoskeletal pain. This way, the present thesis aimed to further disentangle pieces of the complex pathophysiological puzzle underlying chronic INP, chronic WAD, and FM with the ultimate endeavor to increase our understanding of these conditions, and to pave the way for the development of more effective treatment approaches for these patients.

To tackle this core aim seven studies were performed, enclosed in three major parts each addressing one overall objective:

- **Part I** addresses the **first overall objective:** to investigate the current evidence regarding relationships between structural and functional brain alterations and clinical pain measures in chronic musculoskeletal pain patients, and regarding structural and functional brain alterations specifically in INP and WAD. This part comprises two chapters including two systematic reviews.
- **Part II** addresses the **second overall objective:** to examine differences in disability, cognitive deficits, indices of central sensitization (CS), and structural brain alterations, and their interrelationships between women with non-traumatic (INP) and traumatic (WAD) chronic neck pain and healthy pain-free women. Part II includes one large case-control study described in three chapters. We hypothesized that the traumatic origin of neck pain in patients with chronic WAD would be associated with more severe deficits compared to patients with chronic INP.
- **Part III** addresses the **third overall objective:** to explore interactions between cognitive performance, cognitive stress, and CS in patients with chronic WAD and FM, thereby studying differences between these chronic pain disorders. This final part consists of two chapters enclosing the results of the baseline case-control comparisons and the intervention part of a randomized crossover study.

Based on the first systematic review we can conclude that moderate evidence exists for relationships between clinical pain measures, and structural and functional brain alterations measured with Magnetic Resonance Imaging (MRI) within regions or networks involved in somatosensory, affective-motivational, and cognitive pain processing in patients with chronic musculoskeletal pain. In addition, we can infer that chronic musculo-skeletal pain is not only mediated by somatosensory processing of pain but there is a shift towards critical involvement of cognitive and motivational-affective pain processing in

regions often part of the limbic system. The second systematic review found some to moderate evidence for structural and functional brain alterations in patients with chronic WAD, and only for functional brain alterations in patients with chronic INP. More evidence exists for brain alterations in patients with chronic WAD compared to patients with chronic INP, suggesting the presence of different underlying mechanisms in both populations.

Furthermore, the results of part II, part III, and additional research data revealed important differences between patients with chronic INP, chronic WAD and FM, providing evidence for CS associated with subjective and objective cognitive deficits only in patients with chronic WAD and FM. Additionally, this research has demonstrated disability including pain-related disability and limitations on health-related quality of life associated with cognitive deficits in patients with chronic WAD and FM, and to a lesser extent in chronic INP patients. Higher disability, more severe cognitive deficits, and more indices of CS were revealed in patients with FM compared to chronic WAD and chronic INP patients. Accordingly, the chronic INP group may be situated somewhere at the beginning of the continuum of chronic musculoskeletal pain, followed by the chronic WAD group, and the FM group may be the far end of this continuum. As such, the more the experienced pain is centrally driven with increasing severity and complexity of associated symptoms, the more a patient is falling at the end of this continuum.

When comparing patients with chronic INP and chronic WAD, distant hyperalgesia and decreased efficacy of conditioned pain modulation were demonstrated in patients with chronic WAD but not in chronic INP patients, indicating the presence of CS, at a group level, only in patients with chronic WAD.

Nevertheless, when subgrouping based on self-reported symptoms of CS, instead of on diagnosis, only the subgroup showing distinct CS symptoms (also comprising women with chronic INP) demonstrated cognitive deficits, distant hyperalgesia, and decreased conditioned pain modulation efficacy compared to healthy controls. In addition, only in this subgroup with considerable CS symptoms associations between more severe cognitive deficits and distant hyperalgesia were found. These findings highlight the need to address the underlying predominant pain mechanism displayed in the individual patient.

Although it could be hypothesized that alterations in regional grey matter volume, cortical thickness, and white matter microstructure are present in patients with chronic WAD due to the trauma and because of CS, but significantly less in patients with chronic INP, this research was lacking. To address the current research gap, the aim of chapters 4 and 5 was to examine alterations in grey matter morphology and white matter microstructure in brain regions or tracts involved in processing of cognition and pain in women with chronic INP and chronic WAD compared to healthy women, measured with MRI. The second aim was to investigate associations between structural brain alterations, and

measures of cognition, pain, and CS in both chronic neck pain groups. Also, possible brain microhemorrhages related to trauma were evaluated.

The results of chapters 4 and 5 have demonstrated abnormalities in white matter microstructure and decreased grey matter morphology in regions associated with pain and cognitive processing without detecting brain microhemorrhages related to trauma in women with chronic WAD compared to women with chronic INP and healthy women. In patients with chronic WAD, decreased regional grey matter volume was associated with more severe cognitive deficits and more signs of CS. In addition, novel evidence is provided for associations between dysfunctional conditioned pain modulation and the degree of regional white matter deficits in chronic WAD. This yields innovative evidence for underlying white matter microstructural correlates of disturbed endogenous pain inhibition. Accordingly, these results emphasize the role of structural brain alterations in patients with traumatic chronic neck pain (WAD) compared to patients with non-traumatic chronic INP. Together, these findings indicate a possible negative mediating role of the trauma in patients with chronic WAD.

We recommend that disability, cognitive deficits, and CS, and their relationships should be evaluated and treated in patients with chronic INP, chronic WAD and FM with the aim of providing more effective and individually focused therapy, which tackles the observed deficits. Based on the results of this thesis, it can be recommended that therapy approaches for chronic WAD and FM should take into account the role of central nervous system neuroplasticity for example with pain neuroscience education, cognitive behavioral therapy, and cognition-targeted exercise therapy. Also, our findings highlight the need to address the underlying predominant pain mechanism displayed in the individual patient, regardless of diagnosis.

Further research is warranted to investigate which therapies can decrease CS, cognitive deficits, and disability in patients with chronic INP, chronic WAD and FM. In addition, we want to emphasize the need for future research to unravel functional brain alterations in both chronic neck pain populations. Furthermore, longitudinal research should examine which therapeutic approaches can reverse the observed structural brain alterations, and should assess if normalization of these brain alterations is associated with less severe clinical dysfunctions.

In conclusion, the results of this dissertation have unraveled important pieces of the complex pathophysiological puzzle underlying chronic INP, chronic WAD and FM, and have uncovered both differences and overlapping similarities between these chronic musculoskeletal pain conditions. The novel findings increase our understanding of these conditions substantially, and could steer further research and contribute to more effective individually tailored therapy approaches.

Nederlandstalige Samenvatting

Nederlandstalige Samenvatting

Nederlandstalige Samenvatting

Het kerndoel van deze doctoraatsverhandeling was het verbeteren van onze kennis over de mechanismen en hun mogelijke onderlinge relaties die onderliggend zijn aan de aanhoudende, complexe en vaak onverklaarde symptomen van patiënten met chronische idiopathische nekpijn (INP), chronische whiplash geassocieerde aandoeningen (WAD) en fibromyalgie (FM). Het kerndoel was het ontrafelen van de verschillen in de aard en de ernst van de onderliggende mechanismen tussen deze drie heterogene condities met chronische musculoskeletale pijn. Als dusdanig heeft dit doctoraat als doel om delen van de complexe pathofysiologische puzzel die onderliggend is aan chronische INP, chronische WAD en FM verder te ontrafelen met als ultieme doel het inzicht in deze aandoeningen te vergroten, verder onderzoek te sturen en uiteindelijk bij te dragen tot het ontwikkelen van meer effectieve behandelstrategieën voor deze patiënten.

Zeven studies werden uitgevoerd om dit kerndoel te behandelen en werden opgenomen in drie grote delen waarbij elk deel één overkoepelend doel behandelt:

- **Deel I** behandelt het **eerste overkoepelende doel**: de huidige evidentie in kaart brengen betreffende de relaties tussen structurele en functionele hersenveranderingen en klinische pijnmetingen bij patiënten met chronische musculoskeletale pijn, en betreffende structurele en functionele hersenveranderingen specifiek bij INP en WAD patiënten. Dit deel bestaat uit twee hoofdstukken die elk een systematische review bevatten.
- **Deel II** behandelt het **tweede overkoepelende doel**: de verschillen onderzoeken in disfuncties, cognitieve problemen, tekenen van centrale sensitisatie (CS), en structurele hersenveranderingen, alsook hun onderlinge relaties tussen vrouwen met niet-traumatische (INP) en traumatische (WAD) chronische nekpijn en gezonde pijnvrije vrouwen. Deel twee bevat 1 grote case-controle studie beschreven in drie verschillende hoofdstukken. Onze hypothese was dat de traumatische oorsprong van de nekpijn bij patiënten met chronische WAD geassocieerd zou zijn met meer ernstige problemen in vergelijking met chronische INP patiënten.
- **Deel III** behandelt het **derde overkoepelende doel**: de interacties tussen cognitieve prestatie, cognitieve stress en CS exploreren bij patiënten met chronische WAD en FM. Hiermee onderzoeken we verschillen tussen twee chronische pijnaandoeningen die gekenmerkt worden door het gemeenschappelijk pathofysiologisch mechanisme van CS (dit is een overgevoeligheid van het centrale zenuwstelsel). Dit laatste deel bestaat uit twee hoofdstukken die de resultaten bevatten van de baseline case-controle vergelijkingen en het interventie deel van een gerandomiseerde cross-over studie.

Gebaseerd op de resultaten van de eerste systematische review kunnen we concluderen dat er matige evidentie is voor relaties tussen klinische pijnmetingen zoals pijnduur en

pijnintensiteit en structurele en functionele hersenveranderingen gemeten met magnetische resonantie beeldvorming in hersenregio's of netwerken die betrokken zijn bij somatosensorische, emotioneel-motivationele en cognitieve verwerking van pijn bij patiënten met chronische musculoskeletale pijn. Bovendien kunnen we besluiten dat chronische musculoskeletale pijn niet enkel gemedieerd wordt door somatosensorische verwerking van pijn maar dat er een verschuiving plaatsvindt naar een cruciale betrokkenheid van cognitieve en motivationele-emotionele pijnverwerking in hersengebieden die vaak een onderdeel zijn van het limbisch systeem. De tweede systematische review heeft beperkte tot matige evidentie aangetoond voor structurele en functionele hersenveranderingen bij patiënten met chronische WAD en enkel voor functionele hersenveranderingen bij patiënten met chronische INP. Bijgevolg is er meer bewijs aanwezig voor hersenveranderingen bij patiënten met chronische WAD in vergelijking met chronische INP. Deze resultaten suggereren dat er verschillende onderliggende pathofysiologische mechanismen aanwezig zijn bij de beide patiëntengroepen.

Verder hebben de resultaten van deel II en deel III en een bijkomende studie belangrijke verschillen onthuld tussen patiënten met chronische INP, chronische WAD en FM waarbij CS geassocieerd met subjectieve en objectieve cognitieve problemen werd aangetoond enkel bij patiënten met chronische WAD en FM. Bijkomend heeft dit onderzoek disfuncties aangetoond waaronder pijn gerelateerde disfuncties en beperkingen in gezondheidsgerelateerde kwaliteit van leven geassocieerd met cognitieve problemen bij patiënten met chronische WAD en FM en in mindere mate bij patiënten met chronische INP. Meer disfunctie, meer ernstige cognitieve problemen en meer tekenen van CS werden aangetoond bij patiënten met FM in vergelijking met patiënten met chronische WAD en chronische INP. Bijgevolg kan de chronische INP groep gesitueerd worden ergens in het begin van het continuüm van chronische musculoskeletale pijn gevolgd door de chronische WAD groep en de FM groep kan mogelijk gesitueerd worden op het einde van dit continuüm.

Wanneer patiënten met chronische INP en chronische WAD werden vergeleken, werden veralgemeende hyperalgesie en verminderde efficiëntie van geconditioneerde pijnmodulatie (pijn-inhibeert-pijn mechanisme) aangetoond bij patiënten met chronische WAD maar niet bij chronische INP patiënten wat wijst op de aanwezigheid van CS op groepsniveau enkel bij chronische WAD patiënten.

Echter, wanneer subgroepen gebaseerd op zelfgerapporteerde symptomen van CS werden vergeleken in plaats van groepen op basis van diagnose, vertoonde enkel de subgroep met uitgesproken CS symptomen (ook bestaande uit vrouwen met chronische INP) cognitieve problemen, veralgemeende hyperalgesie en verminderde efficiëntie van geconditioneerde pijnmodulatie in vergelijking met gezonde personen. Verder werden er enkel in de subgroep met aanzienlijke symptomen van CS associaties aangetoond tussen meer ernstige cognitieve problemen en veralgemeende hyperalgesie.

Hoewel verondersteld kan worden dat veranderingen in grijze stof volume, corticale dikte, en witte stof microstructuur aanwezig zijn bij patiënten met chronische WAD ten gevolge van het trauma en CS maar significant minder te verwachten zijn bij patiënten met chronische INP, was onderzoek naar hersenveranderingen bij deze patiëntenpopulaties zeer beperkt aanwezig. Bijgevolg was het doel van hoofdstuk 4 en 5 om veranderingen in grijze stof morfologie en witte stof microstructuur in hersenregio's en banen betrokken bij de verwerking van pijn en cognitie te onderzoeken bij vrouwen met chronische INP en chronische WAD ten opzichte van gezonde vrouwen met behulp van magnetische resonantie beeldvorming. Het tweede doel was het onderzoeken van de relaties tussen structurele hersenveranderingen enerzijds en aspecten van cognitie, pijn en CS anderzijds in beide patiëntengroepen met chronische nekpijn. Ook werd de mogelijke aanwezigheid van microbloedingen in de hersenen gerelateerd aan het trauma geëvalueerd.

De resultaten van hoofdstukken 4 en 5 hebben abnormaliteiten in de witte stof microstructuur en gedaalde grijze stof morfologie aangetoond in hersenregio's betrokken bij de verwerking van pijn en cognitie bij patiënten met chronische WAD ten opzichte van chronische INP patiënten en gezonde controle personen. Microbloedingen in de hersenen gerelateerd aan het trauma werden niet gevonden. Er werden geen structurele hersenveranderingen geobserveerd bij chronische INP patiënten ten opzichte van gezonde personen. Bij de chronische WAD patiënten werden er interessante relaties aangetoond tussen gedaald grijze stof volume in pijn en cognitieve verwerkingsgebieden en meer ernstige cognitieve problemen en tekenen van CS. Eveneens werd er innovatief bewijs geleverd voor associaties tussen disfunctionele conditionerende pijnmodulatie en regionale witte stof abnormaliteiten bij chronische WAD patiënten. Dit resultaat levert innovatief bewijs voor onderliggende microstructurele witte stof correlaten van verstoorde endogene pijninhibitie bij patiënten met chronische WAD. Bijgevolg benadrukken deze resultaten de rol van structurele hersenveranderingen bij patiënten met chronische WAD ten opzichte van chronische INP. Deze resultaten wijzen op een mogelijke negatieve mediërende rol van het whiplashtrauma bij patiënten met chronische WAD.

Gebaseerd op de resultaten van deze thesis kunnen verschillende **klinische implicaties** worden gegeven. We bevelen aan dat disfuncties, cognitieve problemen en CS, en hun onderlinge relaties geëvalueerd en behandeld worden bij patiënten met chronische INP, chronische WAD en FM. Zodoende kan meer effectieve en individueel gerichte therapie die inspeelt op de aangetoonde problemen worden toegepast. Verder kan worden aanbevolen dat de therapie van patiënten met chronische WAD en FM de neuroplasticiteit van het centrale zenuwstelsel in rekening brengt bijvoorbeeld door het geven van pijn neurowetenschappelijke educatie, cognitieve gedragstherapie, en cognitie gerichte oefentherapie. Ook benadrukken onze resultaten het belang om in de klinische praktijk het onderliggend dominant aanwezige pijnmechanisme te herkennen en te behandelen bij de individuele patiënt ongeacht de diagnose.

Belangrijke aanbevelingen voor **toekomstig onderzoek** kunnen geformuleerd worden op basis van dit doctoraat. Verder onderzoek is noodzakelijk om na te gaan welke therapieën CS, cognitieve problemen en disfuncties kunnen verminderen bij patiënten met chronische INP, chronische WAD en FM. Verder willen we benadrukken dat het interessant is voor toekomstig onderzoek om functionele hersenveranderingen verder te ontrafelen bij chronische INP en chronische WAD patiënten. Verder longitudinaal onderzoek kan worden aanbevolen met als doel te onderzoeken welke therapeutische strategieën de geobserveerde structurele hersenveranderingen kunnen omkeren, en of de normalisatie van deze hersenveranderingen gepaard gaat met gedaalde klinische disfuncties.

Tenslotte hebben de resultaten van dit doctoraat bijgedragen tot het verder ontrafelen van de complexe pathofysiologische puzzel die onderliggend is aan chronische INP, chronische WAD en FM. Bijkomend werden belangrijke verschillen alsook overlappende gelijkenissen aangetoond tussen deze drie patiëntengroepen met chronische musculoskeletale pijn. Deze nieuwe inzichten leiden tot een belangrijke vergroting van onze kennis over deze pijncondities en kunnen verder onderzoek gericht sturen en leiden tot meer effectieve en geïndividualiseerde therapie voor deze patiënten.

Nederlandstalige Samenvatting

Appendices

Additional research data
List of abbreviations
List of publications
Curriculum vitae

"What we learn with pleasure we never forget."
- A. Mercier

Additional research data

Subgroups based on the central sensitization inventory in women with chronic whiplash and chronic idiopathic neck pain show differences in disability, cognitive deficits, and hyperalgesia

Coppieters I. MSc[1, 2]; Meeus M. PhD[1, 2, 3,*]; De Pauw R. MSc[1]; Kregel J. MSc[1, 2, 4]; Malfliet A. MSc[1, 2, 4]; Danneels L. PhD[1]; Lenoir D. MSc[1]; Cagnie B. PhD[1,*]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium;
[2] Pain in Motion international research group, www.paininmotion.be;
[3] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium;
[4] Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Faculty of Physical Education & Physiotherapy, Free University Brussels, Belgium.
*Equally contributed last authors.

Manuscript in preparation I
Abstract accepted for presentation at the 10th Congress of the European Pain Federation (EFIC), September 2017.

Appendices

Abstract

Background and aims: To date, inconclusive evidence exists regarding the presence of central sensitization (CS) in patients with chronic idiopathic neck pain (CINP) [1]. On the contrary, compelling evidence has demonstrated CS in patients with chronic whiplash associated disorders (CWAD) [2]. Clinically, both differences and overlapping similarities are shown between CINP and CWAD patients [3]. Efficacy of treatment response is variable in these conditions, which possibly originates from the focus on diagnosis when treating CINP and CWAD. Therefore, it could be hypothesized that addressing the underlying predominant pain mechanism in the individual patient is warranted. This study aimed to identify subgroups based on the central sensitization inventory (CSI), a questionnaire to assess self-reported CS symptoms, in a patient group including both women with CINP and CWAD, distinguishing a group with and without distinct self-reported CS symptoms. Subsequently, these subgroups were compared with respect to pain, disability, cognitive deficits, other CS measures, and compared to healthy controls. Furthermore, associations between these features were explored.

Methods: Ninety-five women (28 pain-free controls, 35 CINP, 32 CWAD) completed questionnaires to investigate pain-related disability and health-related quality of life. Next, patients reported neck pain intensity using the verbal numeric rating scale. The CSI was administered to examine self-reported CS symptoms, whereby a score of 40 of 100 distinguishes between a group of central sensitivity syndrome (CSI ≥ 40/100) and a group of non-central sensitivity syndrome patients [4]. To investigate CS experimentally, pressure pain thresholds, and conditioned pain modulation (CPM) efficacy were examined. In addition, self-reported and objective cognitive deficits were assessed. Two subgroups based on the CSI cut-off were defined within the group of CINP and CWAD patients. Differences between both subgroups, and differences with controls were examined for all assessed variables. Finally, spearman correlations were explored within both subgroups between pain and CS measures, and disability and cognitive deficits.

Results: The subgroup reporting CS symptoms indicative of CS (n= 38) displayed higher pain intensity, disability, subjective and objective cognitive deficits, and local hyperalgesia compared to the subgroup reporting symptoms not indicative of CS (n= 24) (p<0.017) (**fig. 1, 2, 3, 4**). Compared to controls, only the subgroup showing distinct CS symptoms demonstrated cognitive deficits, distant hyperalgesia, and decreased CPM efficacy (p<0.017) (**fig. 3, 4, 5**). In addition, moderate correlations were revealed between higher pain intensity, local and distant hyperalgesia, and worse self-reported and objective cognitive performance only in the subgroup presenting considerable CS symptoms (p<0.01) (**table 2**).

Conclusions: This study provides novel evidence for subgroups based on the CSI within CINP and CWAD patients, revealing both heterogeneity and overlap in clinical presentation in both conditions, highlighting the need to address the underlying predominant pain mechanism displayed in the individual patient. Moreover, worse cognitive performance was associated with experimental signs of CS only in the subgroup with indication of CS. These results may steer further research and contribute to the development of more effective therapy approaches.

Key words: chronic whiplash associated disorders, chronic idiopathic neck pain, central sensitization inventory, central sensitization, subgroups, cognitive deficits, disability

Table 1 Demographic characteristics of the healthy pain-free controls, and the CSI-based subgroups including patients with CINP and CWAD.

	CON (n=28)	CINP and CWAD (n= 24) (CSI < 40/100)	CINP and CWAD (n= 38) (CSI ≥ 40/100)	*p* value group test	*p* value post hoc pairwise comparisons		
					CON - CSI < 40/100	CON - CSI ≥ 40/100	CSI < 40/100 - CSI ≥ 40/100
Age (y)[b]	25.5 (23 - 42)	35.5 (24 -43)	36 (27.5 – 45.25)	0.119		NA	
BMI (kg/cm²)[b]	21.83 (20.62 – 23.33)	23.79 (20.80 – 26.81)	22.31 (19.35 – 23.92)	0.109		NA	
CSI (/100)[a]	20.65 (6.78)	32.42 (6.30)	51.40 (8.11)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PCS (/52)[b]	10.00 (1 - 18)	10 (4.25 – 15.75)	18.5 (12.75 -24.25)	**<0.001**	0.691	**0.001**	**<0.001**
PVAQ (/80)[b]	35.00 (21 - 39)	37 (26.75 – 46.75)	38 (32.5 – 46.00)	**0.047**	0.193	**0.009**	0.583
Education level (n)[c] No degree; lower secon.; Higher second.; Higher edu.	0; 1; 7; 20	0; 1; 4; 18	1; 2; 11; 24	0.926		NA	

To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.017 level (Bonferroni correction: 0.05/3). [a]= Data which were assumed to be normally distributed were analyzed with the one-way ANOVA test and post-hoc pairwise comparisons were applied using Bonferroni correction. [b]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and the Mann-Whitney U test for post-hoc comparisons. Shapiro-Wilk test $p<0.05$ and visual inspection of the QQ-plot and histogram provided information that the data were not normally distributed. [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. Values are presented as means and SD for continuous data which were normally distributed and values are presented as median and interquartile range for continuous data which were not normally distributed. Values are presented as absolute frequencies for categorical data. Abbreviations: y= years. CON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain, Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, NA= not applicable, CSI= central sensitization inventory, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire.

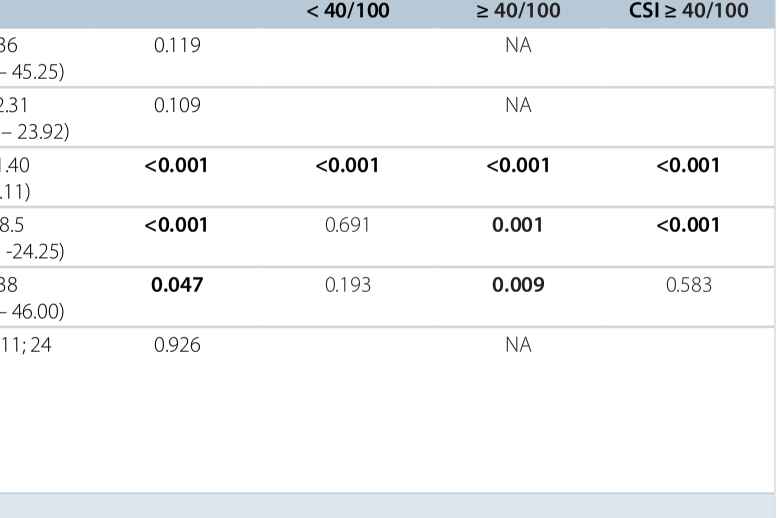


Figure 1 Clinical pain measures in the subgroup reporting symptoms indicative of CS (CSI ≥ 40/100) compared to the subgroup without indication of CS (CSI < 40/100).

n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100. CSI= central sensitization inventory, VNRS= Verbal Numeric Rating Scale, IQR=interquartile range, *=$p<0.05$, **=$p<0.01$. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied.

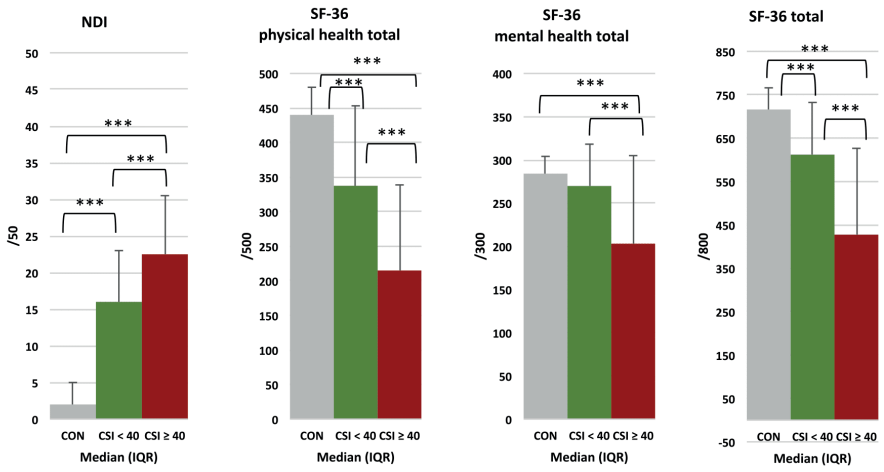


Figure 2 Pain-related disability and health-related quality of life in the subgroup reporting symptoms indicative of CS (CSI ≥ 40/100) compared to the subgroup without indication of CS (CSI < 40/100), and healthy controls.

n= 28 for healthy CON, n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100.
CON= healthy controls, CSI= central sensitization inventory, NDI= Neck Disability Index, SF-36= 36 item short form health survey questionnaire, IQR=interquartile range, ***= $p<0.001$. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied.

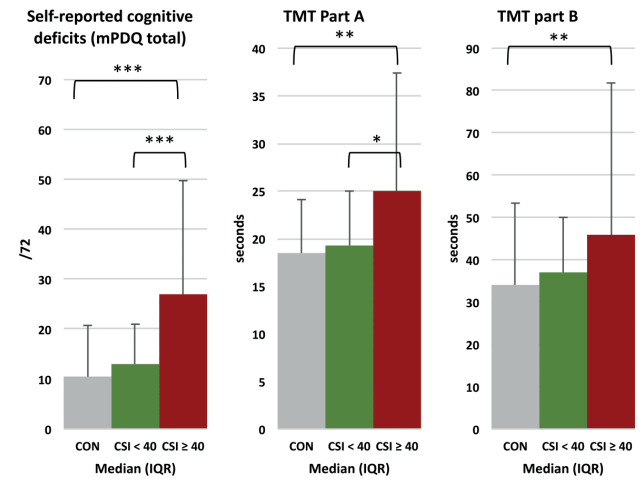


Figure 3 Cognitive performance in the subgroup reporting symptoms indicative of CS (CSI ≥ 40/100) compared to the subgroup without indication of CS (CSI < 40/100), and healthy controls.

n= 28 for healthy CON, n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100.
CON= healthy controls, CSI= central sensitization inventory, mPDQ= modified Perceived Deficits Questionnaire, TMT= trail making test, IQR=interquartile range, *= $p<0.017$ (0.05/3), **=$p<0.01$, ***= $p<0.001$. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied. The mPDQ investigates self-perceived cognitive problems on 4 different cognitive subdomains, i.e. prospective memory, retrospective memory, attention and concentration, and organization and planning. The TMT part A requires mainly visuo-perceptual and processing speed abilities, whereas TMT part B reflects working memory and task-switching ability.

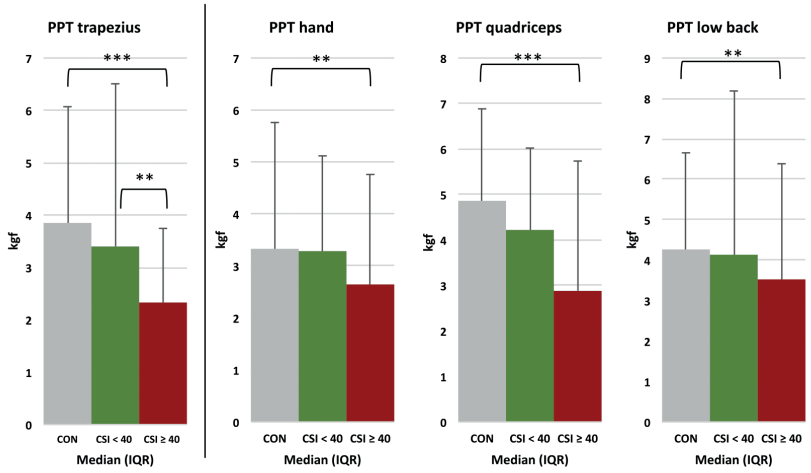


Figure 4 Local and Distant Hyperalgesia in the subgroup reporting symptoms indicative of CS (CSI ≥ 40/100) compared to the subgroup without indication of CS (CSI < 40/100), and healthy controls.

n= 28 for healthy CON, n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100.
CON= healthy controls, CSI= central sensitization inventory, PPT= pressure pain thresholds, IQR=interquartile range, **=p<0.01, ***= p<0.001. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied.

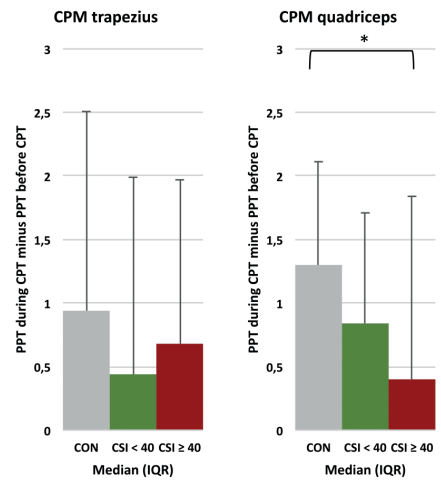


Figure 5 Efficacy of Conditioned Pain Modulation in the subgroup reporting symptoms indicative of CS (CSI ≥ 40/100) compared to the subgroup without indication of CS (CSI < 40/100), and healthy controls.

n= 28 for healthy CON, n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100.
CON= healthy controls, CSI= central sensitization inventory, CPM= conditioned pain modulation, CPT= cold pressor test, PPT= pressure pain thresholds, IQR=interquartile range, *=p<0.017 (0.05/3). Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied.

Table 2 Spearman correlations between cognitive deficits and disability characteristics, and pain intensity and experimental CS measures in patients with CINP and CWAD distinguished based on the CSI cut-off.

CINP and CWAD	Local hyperalgesia	Distant hyperalgesia			CPM efficacy		Clinical pain measure
	PPT trapezius	PPT quadriceps	PPT low back	PPT hand	CPM trapezius	CPM quadriceps	Current neck pain intensity
CSI < 40/100 (n= 24)							
Self-reported cognitive deficits							
mPDQ total	-0,411	-0,024	-0,269	-0,077	0,069	-0,211	0,145
	0,046	*0,913*	*0,204*	*0,721*	*0,755*	*0,334*	*0,498*
Objective cognitive deficits							
TMT part A	-0,132	-0,053	-0,170	-0,325	-0,146	0,096	0,080
	0,539	*0,807*	*0,428*	*0,121*	*0,505*	*0,662*	*0,711*
TMT part B	-0,164	0,301	0,070	-0,063	-0,192	0,011	-0,068
	0,443	*0,153*	*0,747*	*0,771*	*0,381*	*0,961*	*0,752*
Disability characteristics							
NDI	-0,303	-0,266	-0,212	-0,131	-0,165	-0,290	0,444
	0,182	*0,243*	*0,357*	*0,572*	*0,487*	*0,215*	*0,044*
SF-36 total	0,263	-0,040	-0,050	0,160	0,039	0,339	-0,299
	0,215	*0,853*	*0,818*	*0,455*	*0,861*	*0,114*	*0,156*
CSI ≥ 40/100 (n= 38)							
Self-reported cognitive deficits							
mPDQ total	**-0,549**	-0,365	**-0,499**	**-0,582**	-0,330	-0,367	**0,578**
	0,001	*0,029*	***0,002***	***<0,001***	*0,080*	*0,050*	***<0,001***
Objective cognitive deficits							
TMT part A	-0,397	-0,413	-0,411	**-0,540**	-0,243	-0,221	0,370
	0,015	*0,011*	*0,012*	***0,001***	*0,195*	*0,240*	*0,024*
TMT part B	-0,167	-0,242	-0,261	-0,324	-0,018	0,104	0,420
	0,323	*0,149*	*0,118*	*0,051*	*0,923*	*0,586*	*0,010*
Disability characteristics							
NDI	-0,150	-0,266	-0,369	-0,344	-0,069	-0,058	0,314
	0,383	*0,117*	*0,027*	*0,040*	*0,723*	*0,764*	*0,062*
SF-36 total	0,289	0,326	0,319	0,410	0,306	0,012	**-0,464**
	0,078	*0,045*	*0,051*	*0,010*	*0,094*	*0,949*	***0,003***

To correct for multiple comparisons, correlations significant at a statistical threshold level of $p < 0.01$ level (2-tailed) were deemed significant, and are presented in bold and in green. *p*-values are presented below the correlation coefficient. Abbreviations: CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, NDI= neck disability index, SF-36= 36 item short form health survey questionnaire, mPDQ= modified perceived deficits questionnaire, TMT= trail making test, PPT= pressure pain thresholds, CPM= conditioned pain modulation. n= 67 chronic neck pain patients (35 CINP, 32 CWAD) (5 missings for CSI). n= 18 CINP and 6 CWAD for CSI < 40/100, n= 15 CINP and 23 CWAD for CSI ≥ 40/100.

References

1. Malfliet A, Kregel J, Cagnie B, Kuipers M, Dolphens M, Roussel N, Meeus M, Danneels L, Bramer WM, Nijs J. Lack of evidence for central sensitization in idiopathic, non-traumatic neck pain: a systematic review. *Pain Physician* 2015; 18:223-236.
2. Van Oosterwijck J, Nijs J, Meeus M, Paul L. Evidence for central sensitization in chronic whiplash: a systematic literature review. *Eur J Pain* 2013; 17:299-312.
3. Coppieters I., De Pauw R., Kregel J., Malfliet A., Goubert D., Lenoir D., Cagnie B., M. M. Differences between women with traumatic and idiopathic chronic neck pain and women without neck pain: interrelationships among disability, cognitive deficits, and central sensitization. *Physical Therapy* March 2017.
4. Neblett R, Cohen H, Choi Y, Hartzell MM, Williams M, Mayer TG, Gatchel RJ. The Central Sensitization Inventory (CSI): establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. *J Pain* 2013; 14:438-445.

Unravelling the continuum of chronic musculoskeletal pain in women with idiopathic or traumatic chronic neck pain, and fibromyalgia: the role of cognitive deficits and central sensitization

Coppieters I. MSc[1, 2]; Cagnie B. PhD[1]; Schumacher C. MSc[1]; Lenoir D. MSc[1]; Brusselmans G. MD[3]; Ickmans K. PhD[4]; Kregel J. MSc[1, 2, 4]; Malfliet A. MSc[2, 4]; Meeus M. PhD[1, 2, 5]

[1] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium;
[2] Pain in Motion international research group, www.paininmotion.be;
[3] AZ Sint-Lucas Pain clinic, Ghent, Belgium;
[4] Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Faculty of Physical Education & Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium;
[5] Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium.

Manuscript in preparation II.
Abstract accepted for presentation at the 10th Congress of the European Pain Federation (EFIC), September 2017.

Appendices

Abstract

Background and aims: It can be hypothesized that both overlapping and different underlying mechanisms exist between patients with chronic idiopathic neck pain (CINP), chronic whiplash-associated disorders (CWAD) and fibromyalgia (FM). Nevertheless, research investigating differences in underlying mechanisms between these chronic musculoskeletal pain conditions remains scarce. Therefore, the first aim of this study was to examine differences between patients with CINP, CWAD and FM compared to healthy controls regarding cognitive deficits, central sensitization (CS) symptoms, pressure hyperalgesia, and efficacy of conditioned pain modulation (CPM). The second aim was to explore associations between cognitive performance and measures of CS in all study groups.

Methods: Eighty-six women (25 healthy pain-free controls, 18 CINP, 12 CWAD, and 31 FM patients) were included. First, the modified perceived deficits questionnaire was administered to assess self-reported cognitive deficits related to attention, memory and organization. Subsequently, participants performed the Stroop task, psychomotor vigilance task (PVT), and operation span task (OSPAN) to examine objective cognitive performance encompassing selective and sustained attention, and working memory, respectively. Malingering was excluded based on the Rey 15-item memory test. Next, all participants completed the CS inventory (CSI) to examine self-reported symptoms of CS (≥ 40/100). Finally, pressure hyperalgesia and efficacy of CPM were examined to experimentally investigate the presence of CS.

Results: Self-reported cognitive deficits were higher in all patient groups compared to controls (p<0.0125) (**fig. 1**). FM patients reported more severe cognitive deficits compared to CINP patients (p=0.005). Worse objective cognitive performance was revealed in patients with CINP (PVT lapses), CWAD (Stroop, PVT) and FM (Stroop, PVT, OSPAN) compared to controls (p<0.0125) (**fig. 1**). Furthermore, only CWAD and FM patients reported average CSI scores higher than 40 of 100, indicating significant self-reported signs of CS, with FM patients reporting more extensive CS symptoms compared to CWAD (p=0.001) (**fig. 3**). Bilateral widespread pressure hyperalgesia at the quadriceps muscle, lumbar region and calf was demonstrated in patients with CWAD and FM compared to controls (p<0.0125), but not in CINP patients (**fig. 2**). CPM efficacy was comparable between all groups (**fig. 3**). In addition, moderate to strong Spearman correlations were found between more severe self-reported and objective cognitive deficits, and more self-reported and experimental signs of CS in all patient groups (p<0.05) (**table 2**).

Conclusions: The present study revealed important differences between women with CINP, CWAD and FM compared to healthy women, providing evidence for CS associated with cognitive deficits in patients with CWAD and FM. In CINP patients, CS could not be demonstrated at group level, while cognitive deficits were present, however, to a lesser extent compared with CWAD and FM patients. The CINP group may be situated somewhere at the beginning of the continuum of chronic musculoskeletal pain, followed by the CWAD group, and the FM group may be the far end of this continuum. As such, the more the experienced pain is centrally driven with increasing severity and complexity of associated symptoms, the more a patient is falling at the end of the chronic musculoskeletal pain continuum.

Key words: chronic idiopathic neck pain, chronic whiplash associated disorders, fibromyalgia, cognitive performance, central sensitization, pressure hyperalgesia

Table 1 Demographic characteristics of the patients with CINP, CWAD and FM, and healthy pain-free controls (n= 86).

Characteristics	CON (n=25)	CINP (n=18)	CWAD (n=12)	FM (n=31)	*p* value Group test	*p* value post hoc pairwise comparisons					
						CON-CINP	CON-CWAD	CON-FM	CINP-CWAD	CWAD-FM	CINP-FM
Age (y)[a]	31,60 (11,05)	41,78 (12,92)	39,58 (12,15)	40,06 (11,12)	**0.018**	0.035	0.325	0.050	1.000	1.000	1.000
Body height (cm)[a]	166,48 (5,60)	167, 61 (6,42)	164,08 (5,88)	166,06 (6,65)	0.500	NA					
Body mass (kg)[a]	61,18 (7,14)	63,53 (8,01)	60,62 (7,81)	68,39 (11,92)	**0.019**	1.000	1.000	0.033	1.000	0.104	0.510
BMI (kg/cm^2)[a]	22,06 (2,21)	22,66 (3,05)	22,50 (2,55)	24,78 (4,12)	**0.012**	1.000	1.000	0.014	1.000	0.244	0.177
Education level (n)[c] No degree; lower secon.; Higher second.;Higher edu.	0; 0; 4; 20	0; 1; 7; 10	0; 0; 3; 8	0; 3; 11; 17	0.274	NA					
PDI (/70)[b]	0,00 (0,00 – 0,00)	7,00 (2,75 – 33,00)	30,00 (21,00 – 44,25)	36,00 (31,00 – 42,00)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.051	0.136	**<0.001**
PCS (/52)[b]	5,00 (0,00 – 15,00)	9,00 (5,75 – 24,25)	13,00 (7,00 – 30,00)	22,00 (18,00 – 31,00)	**<0.001**	0.035	**0.010**	**<0.001**	0.279	0.080	**0.001**
PVAQ (/80)[b]	29,00 (19,00 – 36,50)	33,50 (20,75 – 42,25)	34,00 (29,25 – 53,75)	41,00 (37,00 – 48,00)	**<0.001**	0.213	0.062	**<0.001**	0.409	0.249	**0.008**
HADS (/42)[b]	5,00 (1,00 – 9,00)	10,00 (7,75 – 14,75)	8,50 (5,25 – 20,25)	18,00 (15,00 – 21,00)	**<0.001**	**0.001**	**0.012**	**<0.001**	0.766	0.043	**<0.001**
Malingering (Rey-15 <9/15) (yes/no)	(0/25)	(0/18)	(0/12)	(0/31)		NA					

To correct for multiple comparisons, differences measured with the Mann-Whitney U test were only deemed significant below the 0.0125 level (Bonferonni correction: 0.05/4). [a]= Data which were assumed to be normally distributed were analyzed with the one-way ANOVA test and post-hoc pairwise comparisons were applied using Bonferroni correction. [b]= Data which were not normally distributed and subsequently group differences were analyzed using the Kruskal-Wallis test and the Mann-Whitney U test for post-hoc comparisons. Shapiro-Wilk test $p<0.05$ and visual inspection of the QQ-plot and histogram provided information that the data were not normally distributed. [c]= categorical data was analyzed by performing the Fisher's exact test. Significant differences were presented in Bold. Values are presented as means and SD for continuous data which were normally distributed and values are presented as median and interquartile range for continuous data which were not normally distributed. Values are presented as absolute frequencies for categorical data.
Abbreviations: y= years. CON= healthy pain-free controls, CWAD= chronic whiplash-associated disorders, CINP= chronic idiopathic neck pain, FM= fibromyalgia. VNRS= verbal numeric rating scale. Lower second= lower secondary, Higher second= higher secondary, Higher edu= higher education, NA= not applicable, PDI= pain disability index, PCS= pain catastrophizing scale, PVAQ= pain vigilance and awareness questionnaire, HADS= hospital anxiety and depression scale.

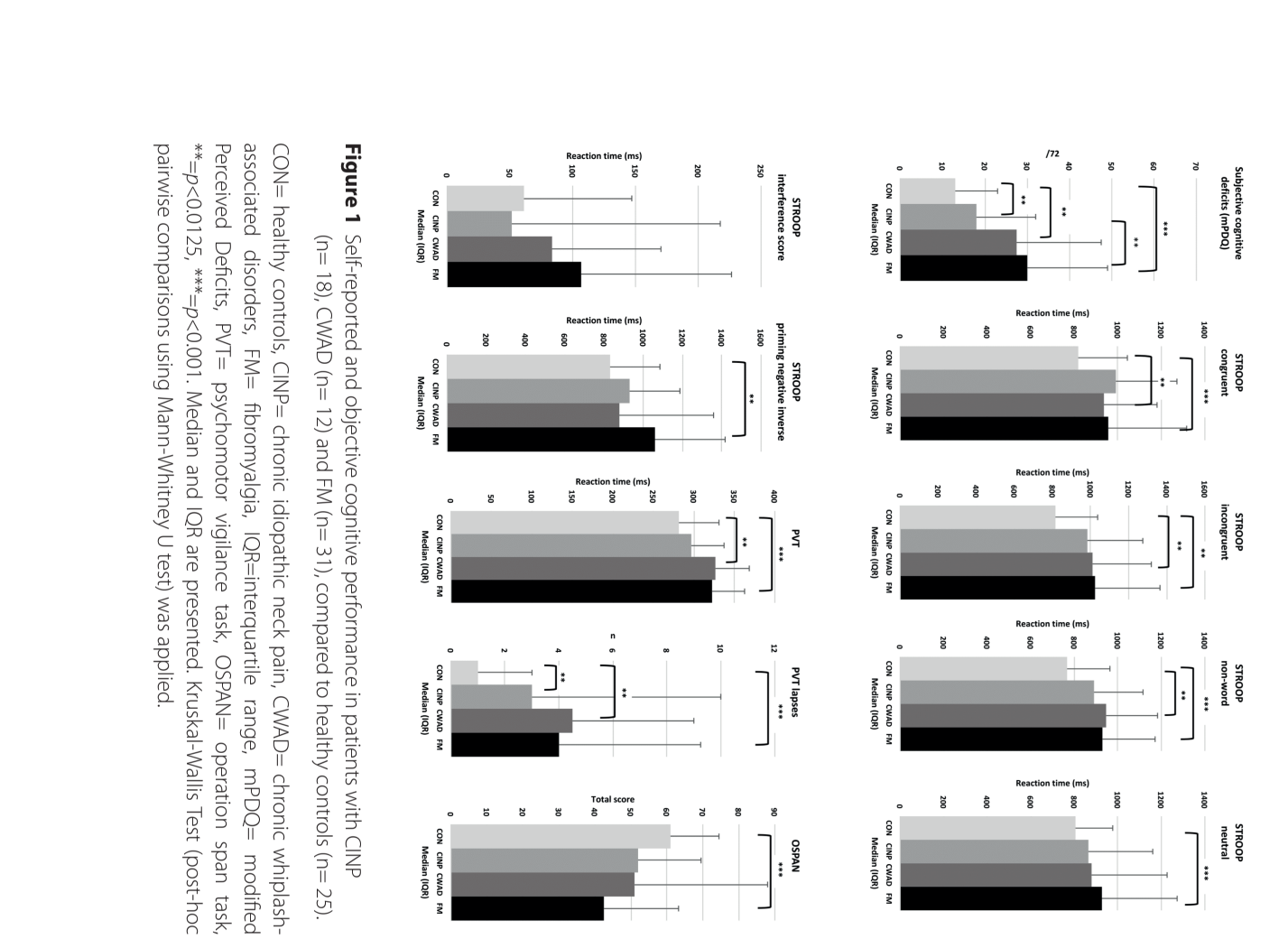


Figure 1 Self-reported and objective cognitive performance in patients with CINP (n= 18), CWAD (n= 12) and FM (n= 31), compared to healthy controls (n= 25).

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, FM= fibromyalgia, IQR=interquartile range, mPDQ= modified Perceived Deficits, PVT= psychomotor vigilance task, OSPAN= operation span task, **=$p<0.0125$, ***=$p<0.001$. Median and IQR are presented. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied.

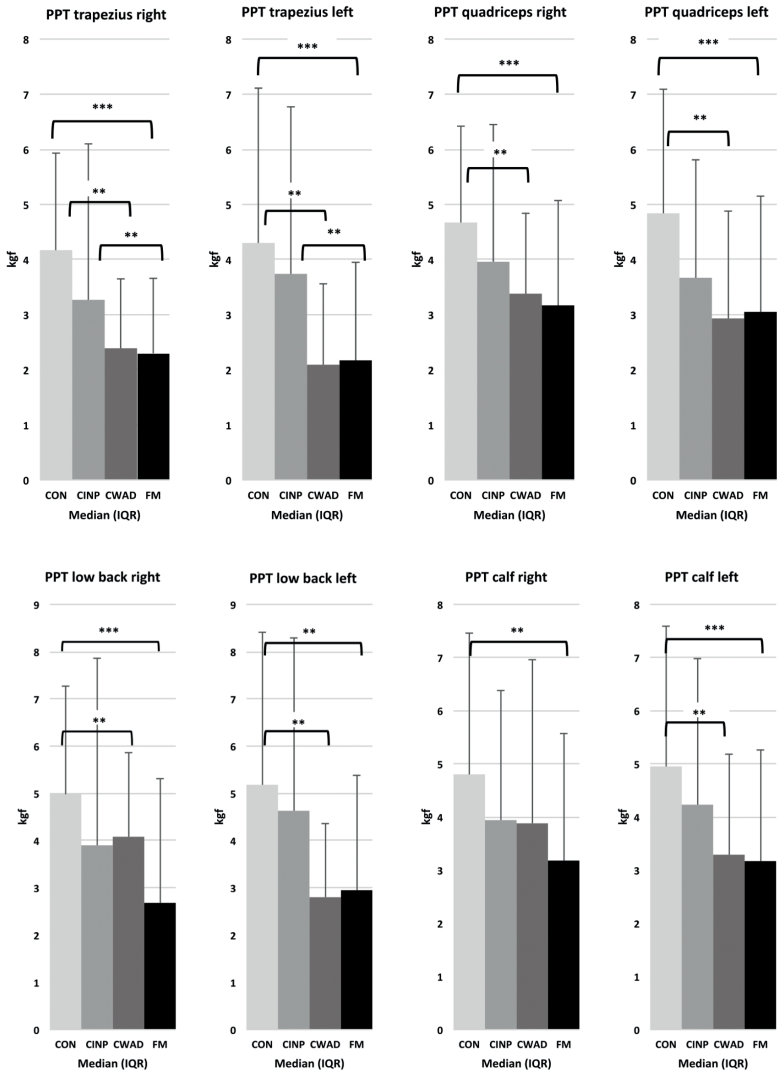


Figure 2 Pressure hyperalgesia in patients with CINP (n= 18), CWAD (n= 12), and FM (n= 31), compared to healthy controls (n= 25).

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, FM= fibromyalgia, PPT= pressure pain thresholds, kgf = kilogram force (1 kgf = 9,81 N), IQR=interquartile range, **=p<0.0125 (0.05/4), ***=p<0.001. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied. Results of experimental measures of central sensitization are presented in particular pressure pain threshold measurements to examine pressure hyperalgesia.

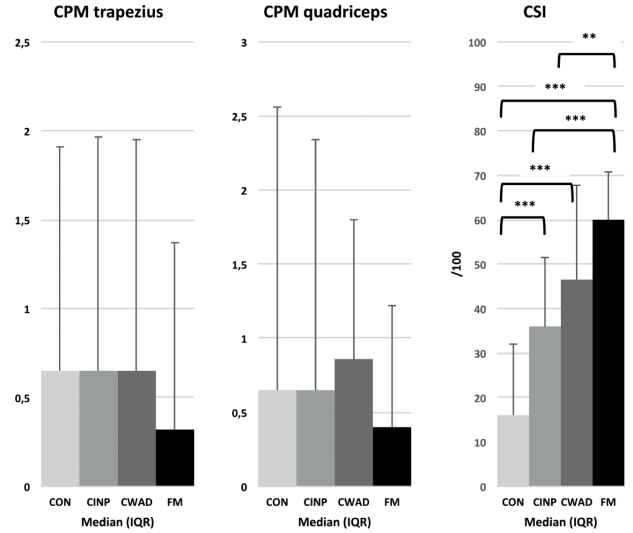


Figure 3 Efficacy of CPM and self-reported symptoms of CS in patients with CINP (n= 18), CWAD (n= 12), and FM (n= 31), compared to healthy controls (n= 25).

CON= healthy controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, FM= fibromyalgia, CPM= conditioned pain modulation (PPT during cold pressor test minus PPT before cold pressor test), kgf = kilogram force (1 kgf = 9,81 N), IQR=interquartile range, **=p<0.0125, ***=p<0.001. Median and IQR are presented. Kruskal-Wallis Test (post-hoc pairwise comparisons using Mann-Whitney U test) was applied. Results of experimental measures of central sensitization are presented, in particular measurements of CPM efficacy at the middle trapezius and at the quadriceps muscle. In addition, results of the central sensitization inventory (CSI) are displayed to investigate self-reported symptoms of central sensitization.

Appendices

Table 2 Spearman correlations between self-reported and objective cognitive performance, pressure hyperalgesia, CPM efficacy and self-reported CS symptoms in patients with CINP, CWAD and FM, and healthy pain-free controls.

	mPDQ total	Stroop RT congruent	Stroop RT incongruent	Stroop RT non-word	Stroop RT neutral	Stroop interference	Stroop RT priming neg. inv	PVT RT	PVT lapses	OSPAN total score
CINP (n= 17)										
PPT trapezius R	-0,330	-0,291	-0,244	-0,202	-0,299	-0,288	-0,249	-0,432	-0,374	-0,302
PPT trapezius L	-0,279	-0,108	-0,105	0,010	-0,088	-0,390	-0,199	-0,346	-0,189	-0,037
PPT quadriceps R	-0,215	-0,137	-0,054	-0,061	-0,012	-0,118	-0,103	-0,337	-0,219	0,191
PPT quadriceps L	-0,131	0,022	0,120	0,135	0,002	-0,208	0,010	-0,327	-0,106	0,140
PPT low back R	-0,371	-0,316	-0,260	-0,154	-0,206	-0,272	-0,321	-0,373	-0,324	0,043
PPT low back L	-0,423	-0,382	-0,319	-0,191	-0,294	-0,326	-0,397	**-0,519***	-0,428	-0,021
PPT calf R	-0,255	-0,353	-0,238	-0,201	-0,211	-0,228	-0,260	-0,422	**-0,506***	-0,127
PPT calf L	-0,250	-0,350	-0,181	-0,181	-0,284	-0,162	-0,248	-0,465	-0,434	-0,096
CPM trapezius	-0,368	-0,259	-0,256	-0,235	-0,276	-0,341	-0,344	-0,471	-0,327	-0,199
CPM quadriceps	-0,337	-0,176	-0,235	-0,015	-0,215	**-0,571***	-0,285	-0,238	-0,120	-0,297
CSI	**0,698****	**0,610****	**0,794*****	**0,594***	**0,642****	**0,566***	**0.715*****	0,267	0,382	-0,139
CWAD (n= 11)										
PPT trapezius R	0,114	-0,296	-0,232	-0,164	-0,323	-0,150	-0,141	-0,196	-0,087	0,543
PPT trapezius L	0,000	**-0,609***	-0,500	-0,482	**-0,645***	-0,509	-0,427	-0,373	-0,514	**0,778****
PPT quadriceps R	-0,200	-0,482	-0,527	-0,536	-0,491	-0,418	**-0,618***	0,100	-0,150	0,310
PPT quadriceps L	**-0,664***	-0,518	-0,600	-0,555	-0,373	-0,355	-0,527	-0,373	-0,238	0,225
PPT low back R	0,169	-0,100	-0,050	-0,082	-0,274	-0,027	-0,142	0,023	-0,099	**0,774****
PPT low back L	0,073	-0,378	-0,255	-0,228	-0,506	-0,387	-0,301	-0,200	-0,391	0,421
PPT calf R	-0,291	-0,600	**-0,655***	**-0,627***	**-0,718***	**-0,627***	**-0,755****	-0,300	-0,365	0,322
PPT calf L	-0,355	**-0,673***	**-0,655***	**-0,727***	**-0,791****	-0,418	**-0,691***	-0,564	**-0,841*****	0,535
CPM trapezius	0,460	-0,025	-0,067	-0,050	-0,285	-0,092	-0,201	0,594	0,306	-0,172
CPM quadriceps	0,212	0,030	-0,091	0,030	-0,164	-0,273	-0,127	0,115	0,496	0,049
CSI	**0,834*****	0,564	0,567	**0,602***	0,452	0,291	0,564	**0,711****	**0,819*****	-0,164
FM (n= 29)										
PPT trapezius R	-0,255	0,099	0,194	0,116	0,173	0,220	0,203	-0,201	-0,335	0,162
PPT trapezius L	-0,148	0,084	0,169	0,118	0,184	0,094	0,133	-0,177	**-0,376***	0,188
PPT quadriceps R	-0,350	0,038	0,118	0,089	0,139	0,152	0,090	**-0,375***	**-0,371***	-0,018
PPT quadriceps L	-0,174	0,08	0,138	0,063	0,152	0,217	0,079	-0,314	-0,336	0,021
PPT low back R	-0,109	0,072	0,195	0,104	0,150	0,262	0,162	-0,314	-0,301	-0,055
PPT low back L	-0,235	0,031	0,134	0,093	0,150	0,117	0,086	-0,240	-0,323	0,086
PPT calf R	-0,293	-0,057	0,026	-0,045	0,033	0,177	-0,005	-0,364	**-0,381***	0,060
PPT calf L	**-0,399***	-0,058	0,011	-0,010	0,027	0,125	-0,068	**-0,373***	**-0,420***	0,129
CPM trapezius	0,138	0,042	0,118	-0,028	0,019	0,183	0,029	-0,220	-0,316	-0,061
CPM quadriceps	0,249	0,145	0,223	0,174	0,195	0,087	0,130	-0,035	-0,100	-0,080
CSI	**0,552*****	**0,559*****	**0,491****	**0,558*****	**0,545***	0,120	**0,442***	**0,412***	**0,399***	-0,201

Significant correlations are presented in bold. *Spearman correlations is significant at the 0.05 level (2-tailed). ** Spearman correlation is significant at or below the 0.01 level (2-tailed). *** Spearman correlation is significant at or below the 0.001 level (2-tailed). FM=fibromyalgia, CWAD= chronic whiplash associated disorders, mPDQ= modified Perceived Deficits Questionnaire. In the healthy control group, the following significant correlations ($p<0.05$) were observed: mPDQ total score correlated with PPT quadriceps R (-0.427) and PPT calf L (-0.478); CPM quadriceps correlated with Stroop RT incongruent (-0.442); PPT calf L correlated with Stroop RT neural (-0.449).

List of abbreviations

Appendices

List of abbreviations

ACC	Anterior cingulate cortex
ACR	American College of Rheumatology
AD	Axial diffusivity
BOLD	Blood oxygen-level dependent
CINP	Chronic idiopathic neck pain
CNS	Central nervous system
CPM	Conditioned pain modulation
CS	Central sensitization
CSI	Central sensitization inventory
CWAD	Chronic whiplash associated disorders
DLPFC	Dorsolateral prefrontal cortex
DMN	Default mode network
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
FA	Fractional anisotropy
FC	Functional connectivity
FM	Fibromyalgia
fMRI	Functional Magnetic Resonance Imaging
GM	grey matter
GMV	Grey matter volume
HPA	Hypothalamus-pituitry-adrenal
IASP	International Association for the Study of Pain
INP	Idiopathic neck pain
LTP	Long-term potentiation
MD	Mean diffusivity
mPDQ	modified Perceived Deficits Questionnaire
MRI	Magnetic Resonance Imaging
MSKP	Musculoskeletal pain
NDI	Neck disability index
NFR	Nociceptive flexion reflex
NK1	Neurokinin 1
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Newcastle-Ottawa Scale
OSPAN	Operation span task
PAG	Periaqueductal grey
PCC	Posterior cingulate cortex
PCS	Pain Catastrophizing Scale
PET	Positron Emission Tomography
PPT	Pressure pain threshold
PRT	Progressive relaxation therapy
PVAQ	Pain Vigilance and Awareness questionnaire
PVT	Psychomotor vigilance task
QoL	Quality of life
QST	Quantitative sensory testing
QTF	Quebec Task Force
rCBF	Regional cerebral blood flow
RD	Radial diffusivity

Appendices

RVM	Rostral ventromedial medulla
S1	Primary somatosensory cortex
SF-36	Short form 36-item health survey
SPECT	Single-Photon Emission Computed Tomography
SS	Symptom severity scale
TBI	Traumatic brain injury
TMT	Trail making test
TS	Temporal summation
TSK	Tampa scale of kinesiophobia
VAS	Visual analogue scale
VBR	Ventricle-brain ratio
vmPFC	Ventromedial prefrontal cortex
VNRS	Verbal numeric rating scale
WAD	Whiplash associated disorders
WM	white matter
WPI	widespread pain index

List of publications

Appendices

List of publications

Publications in journals with peer review

Cagnie B, Dewitte V, **Coppieters I**, Van Oosterwijck J, Cools A, Danneels L. Effect of Ischemic Compression on Trigger Points in the Neck and Shoulder Muscles in Office Workers: a Cohort Study. Journal of manipulative and physiological therapeutics. 2013. 36(8): 482–489. Impact factor 2013: 1.248. Q3 in Integrative and complementary medicine, rank: 12/22.

Meeus M, Goubert D, De Backer F, Stryf F, Hermans L, **Coppieters I**, De Wandele I, Da Silva H, Calders P. Heart Rate Variability in Patients with Fibromyalgia and Patients with Chronic Fatigue Syndrome: a Systematic Review. Seminars in arthritis and rheumatism. 2013. 43(2): 279–287. Impact factor 2013: 3.629, Q2 in Rheumatology rank: 9/30.

Cagnie B*, **Coppieters I***, Denecker S, Six J, Danneels L, Meeus M. Central sensitization in fibromyalgia? A systematic review on structural and functional brain MRI. Seminars in arthritis and rheumatism. 2014. 44(1): 68-75. Peer reviewed journal. Impact factor 2014: 3.925. Q1 in Rheumatology, rank: 8/32. *Equally contributed first authors.

Meeus M, Van Oosterwijck J, Ickmans K, Baert I, **Coppieters I**, Roussel N, Stryf F, Pattyn N, Nijs J. Interrelationships Between Pain Processing, Cortisol and Cognitive Performance in Chronic Whiplash-associated Disorders. Clinical Rheumatology. 2015. 34(3): 545–553. Impact factor 2015: 2.042. Q3 in Rheumatology, rank: 20/32.

Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R Noten S, Meeus M. Cognitive performance is related to central sensitization and health-related quality of life in patients with chronic whiplash-associated disorders and fibromyalgia. Pain Physician. 2015. May-Jun;18(3): 389-401. Impact factor: 3.407. Q2 in Anesthesiology, rank: 8/31, Q2 in Clinical neurology, rank: 53/193.

De Pauw R, **Coppieters I**, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? - A systematic review. Manual Therapy. 2015; 22: 42-49. Impact factor 2015: 1.869. Q1 in Rehabilitation, rank: 15/65.

Coppieters I, Cagnie B, Nijs J, Van Oosterwijck J, Danneels L, De Pauw R, Meeus M. Effects of stress and relaxation on central pain modulation in chronic whiplash and fibromyalgia patients compared to healthy controls. Pain Physician. Mar 2016; 19(3): 119-130. Impact factor: 3.407. Q2 in Anesthesiology, rank: 8/31, Q2 in Clinical neurology, rank: 53/193.

Coppieters I, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. The Journal of Pain. Sep 2016; 17(9): 949-962. Impact factor 2015: 4,463. Q1 in clinical neurology, rank 31/193, Q1 in neurosciences, rank: 56/256.

De Meulemeester K, Castelein B, **Coppieters I**, Barbe T, Cools A, Cagnie B . Differences in outcome between trigger point dry needling and manual pressure technique for the management of myofascial neck/shoulder pain: a randomized clinical trial. Journal of Manipulative and Physiological Therapeutics. 2017 Jan;40(1):11-20. Impact factor 2015: 1.329. Q3 in Rehabilitation, rank 37/65.

Coppieters I, De Pauw R, Kregel J, Malfliet A, Goubert D, Lenoir D, Cagnie B, Meeus M. Differences between women with traumatic and idiopathic chronic neck pain and women without neck pain: interrelationships among disability, cognitive deficits, and central sensitization. Physical Therapy, 2017; 97:338-353. Impact factor 2015: 2.799. Q1 & D1 in Rehabilitation, rank: 5/65.

Kregel J, **Coppieters I**, De Pauw R, Malfliet A, Danneels L, Nijs J, Cagnie B, Meeus M. Does conservative treatment change the brain in patients with chronic musculoskeletal pain? A systematic review. Pain Physician, 2017 Mar; 20(3):139-154. Impact factor: 3.407. Q2 in Anesthesiology, rank: 8/31, Q2 in Clinical neurology, rank: 53/193.

De Pauw R*, **Coppieters I***, Meeus M, Caeyenberghs K, Danneels L, Cagnie B. Is traumatic and non-traumatic neck pain associated with brain alterations? A systematic review. Pain Physician, 2017; 20(4): 245-260. Impact factor 2015: 3.40. Q2 in anesthesiology, rank 8/31.
*Equally contributed first authors.

Malfliet A, **Coppieters I**, Van Wilgen P, Kregel J, De Pauw R, Dolphens M, Ickmans K. Brain changes associated with cognitive and emotional factors in chronic pain: A systematic review. European Journal of Pain. 2017; 21(5):769-786. Impact factor 2015: 2.90, Q2 in Anesthesiology, rank 9/31.

Accepted in journals with peer review

Coppieters I, De Pauw R, Caeyenberghs K, Danneels L, Meeus M*, Cagnie B*. Decreased regional grey matter volume in chronic whiplash-associated disorders: relationships with cognitive deficits and disturbed pain processing. Accepted for publication in Pain Physician, April 2017. Impact factor: 3.407. Q2 in Anesthesiology, rank: 8/31, Q2 in Clinical neurology, rank: 53/193.
*Equally contributed last authors.

Goubert D, Danneels L, Graven-Nielsen T, Descheemaeker F, **Coppieters I**, Meeus M. Differences in pain processing between patients with chronic low back pain, recurrent low back pain and fibromyalgie. Accepted in Pain Physician, November 2016. Impact factor 2015: 3.407, rank 8/31.

Publication letters and short communications

Coppieters I, Malfliet A. Chronic whiplash-associated disorders: reorganization of the brain? Invited commentary paper. Published in EbioMedicine. EBioMedicine (2016) 29–30.

Submitted papers

Coppieters I, De Pauw R, Caeyenberghs K, Lenoir D, Deblaere K, Genbrugge E, Meeus M*, Cagnie B*. Differences in white matter microstructure and cortical thickness between patients with traumatic and idiopathic chronic neck pain: associations with cognition and pain modulation?
*equally contributed last authors. Submitted.

Kregel J, **Coppieters I**, Caeyenberghs K, Malfliet A, Danneels L, Dolphens M, Nijs J, Meeus M, Cagnie B. Brain morphology relates to psychophysiological pain measures in chronic low back pain patients: the role of gender. Submitted in Pain Medicine.

De Pauw R, **Coppieters I**, Palmans T, Danneels L, Meeus M, Cagnie B. Motor impairment in patients with chronic neck pain: does the traumatic event play a significant role? – A case control study. Under review.

Goubert D, Meeus M, Willems T, De Pauw R, **Coppieters I**, Crombez G, Danneels L. The association between structural back muscle characteristics and pain mechanism in low back pain patients. Submitted in The Clinical Journal of Pain. December 2016. Impact factor 2015: 2.712, rank 11/31.

Lenoir D, De Pauw R, Ickmans K, Schumacher C, Timmers I, Kregel J, **Coppieters I**. Validity and test-retest reliability of the Dutch modified Perceived Deficits Questionnaire to examine cognitive symptoms in women with chronic whiplash, chronic idiopathic neck pain and fibromyalgia. Submitted.

Kregel J, Schumacher C, Dolphens M, Malfliet A, Goubert D, Lenoir D, Cagnie B, Meeus M, **Coppieters I**. Validity of the central sensitization inventory: association with experimental pain measures, quality of life, disability, and pain cognitions. Submitted.

Appendices

Published Abstracts

Belgian Back Society (BBS) Congress. The effects of neurocognitive stress and relaxation on central pain processing in patients with chronic whiplash associated disorders and healthy controls: a randomized controlled trial. November 2014. **Coppieters I**, Cagnie B, Nijs J, van Oosterwijck J, Danneels L, De Pauw R, Meeus M.

Pain Science in Motion Congress. Cognitive performance is related to central sensitization in patients with chronic whiplash associated disorders and fibromyalgia: A case-control study. *Runner-up for the prize of best abstract* at the Pain Science in Motion International and Interdisciplinary Colloquium on Research Methods in Pain Science in Brussels, Belgium. March 2015. **Coppieters I**, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M.

European pain Federation (EFIC) congress in Vienna. Central pain modulation and cognitive functioning in patients with traumatic and non-traumatic chronic neck pain: preliminary results. September 2015. **Coppieters I**, Cagnie B, De Pauw R, Danneels L, Ickmans K, Meeus M.

IFOMPT Conference in Glasgow. July 2015. Differences between traumatic and non-traumatic chronic neck pain patients: the role of central pain modulation, cognitive functioning and psychosocial characteristics. **Coppieters I**, Cagnie B, De Pauw R, Danneels L, Ickmans K, Meeus M.

IFOMPT Conference in Glasgow. July 2016. Altered grey matter morphology of pain processing regions in traumatic and non-traumatic neck pain patients: relations with clinical pain measures? **Coppieters I**, Meeus M, De Pauw R, Caeyenberghs K, Danneels L, Cagnie B.

IFOMPT Conference in Glasgow. July 2016. Associations between brain morphological findings and pain measures in chronic spinal pain: preliminary baseline results of a randomized controlled trial.
Kregel J, **Coppieters I**, Malfliet A, Dolphens M, Roussel N, Caeyenberghs K, Danneels L, Meeus M, Nijs J, Cagnie B.

Pain Science in Motion Congress in Stockholm. March 2017. Decreased regional grey matter volume in chronic whiplash associated disorders: relationships with cognitive deficits, pain and central sensitization. **Coppieters I**, De Pauw R, Caeyenberghs K, Danneels L, Kregel J, Meeus M*, Cagnie B*. *Equally contributed last authors. Pain Reports 2(1):e583, January-February 2017.

Curriculum vitae

Curriculum vitae

General information

Name: Iris Coppieters
Birth date: November 13th, 1989
Place of birth: Brasschaat, Belgium
Nationality: Belgian
E-mail: iris.coppieters@ugent.be

Professional experience

2013-Present Assistant-researcher (100%) at the department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium.

Research in the field of chronic pain focusing on structural and functional brain imaging, central pain processing, experimental pain measurements, cognitive functioning, and psychosocial measures.

Teaching experience

2013-Present Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent University, Belgium.

Scientific Project in Rehabilitation Sciences and Physiotherapy – 3rd Bachelor.
Screening and evaluation – Practicum - 1st Master Major Musculoskeletal Physiotherapy.
Teaching (conducting a systematic review & Screening and evaluation -1st Master Major Musculoskeletal Physiotherapy).
Supervision Master thesis students (1st Master and 2nd Master).
3rd Bachelor: Supervising systematic literature study.
2nd and 3rd Bachelor: Supervision Student of the Honours Programme in Life Sciences.

30-11-2016 – 1-12-2016 Scholing Randstad West. Goes. Nederland. Whiplash: een clash tussen nek en brein? Prof. dr. Barbara Cagnie. Prof. dr. Mira Meeus. drs. Iris Coppieters.

Research experience

2011-2013 Dysfunctions of the low back muscles in relation to disturbed central pain mechanisms: recurrent versus chronic low back pain: a pilot study (Master thesis).

2013-Present Relationships between cognitive deficits, central sensitization, and structural brain alterations in patients with chronic idiopathic neck pain, chronic whiplash associated disorders and fibromyalgia: *unravelling differences in underlying mechanisms* (PhD. thesis).

2013 – Present Research domains: chronic pain, cognitive performance, structural and functional brain alterations, central sensitization, experimental pain measurements, pain neurophysiology, physiotherapy, Brain Magnetic Resonance Imaging, FreeSurfer analysing techniques, ExploreDTI analysing techniques.

Administrative functions, Memberships and activities in University

2013-Present Member of the research group "Pain in Motion", International research group of Pain researchers (www.paininmotion.be).

Member of the research group "Spine", research group of researchers specialized in neuropathophysiology and rehabilitation of (chronic) spinal pain patients. Department of Rehabilitation Sciences and Physiotherapy. Faculty of Medicine and Health sciences, Ghent University, Belgium.

2015-Present Member of the education committee at the Department of Rehabilitation Sciences and Physiotherapy. Faculty of Medicine and Health sciences, Ghent University, Belgium.

2016-Present Member and secretary of the Steering Committee of the "Pain in Motion group", International research group of Pain researchers (www.paininmotion.be).

2016-2017 Member of the congress scientific committee "Pain Science in Motion Congress" organized by Pain in Motion. Karolinska Institut, Stockholm, Sweden. March 24-25, 2017.

Referee work

2015-Present Reviewer for international journals: Reviewer for the journal Physiotherapy theory and Practice, Reviewer for the journal Comprehensive Psychiatry, Reviewer for the journal Neuroimage: clinical, Reviewer for the journal Brain Research, Reviewer for the Journal EBioMedicine, Reviewer for the journal Pain Practice.

Research awards

November 2014 **Young Investigator Award**, The Belgian Back Society, Abstract submission and poster presentation at the 7th International congress of the Belgian Back Society "Neck Pain, a 360° view", Ghent, Belgium: The effects of neurocognitive stress and relaxation on central pain processing in patients with chronic whiplash associated disorders and healthy controls: a randomized controlled trial. Coppieters I, Cagnie B, Nijs J, van Oosterwijck J, Danneels L, De Pauw R, Meeus M.

September 2015 **Best Scientific Poster Award**, Manual Therapy Association Belgium, Poster presentation at the European congress of Manual therapy in Louvain. Altered grey matter morphology of pain processing regions in chronic whiplash and chronic idiopathic neck pain patients. Coppieters I, De Pauw R, Caeyenberghs K, Cagnie B, Danneels L, Meeus M.

March 2015 **Runner-up for the prize of best abstract** at the Pain Science in Motion International and Interdisciplinary Colloquium on Research Methods in Pain Science in Brussels, Belgium. Pain Science in Motion Congress. 'Cognitive performance is related to central sensitization in patients with chronic whiplash associated disorders and fibromyalgia: A case-control study'. Coppieters I, Ickmans K, Cagnie B, Nijs J, De Pauw R, Noten S, Meeus M.

March 2017 **Award for best oral presentation** at the Pain Science in Motion International and Interdisciplinary Colloquium on Research Methods in Pain Science in Stockholm, Sweden. Structural brain alterations in women with chronic whiplash associated disorders: relationships with cognitive deficits and disturbed pain processing. Coppieters I, De Pauw R, Caeyenberghs K, Danneels L, Kregel J, Cagnie B, Meeus M.

International Presentations

Oral Presentation. Pain Science in Motion Congress. Cognitive performance is related to central sensitization in patients with chronic whiplash associated disorders and fibromyalgia: A case-control study' at the Pain Science in Motion International and Interdisciplinary Colloquium on Research Methods in Pain Science in Brussels, Belgium. March 26-27, 2015. **Coppieters I**, Ickmans K, Cagnie B, Nijs J, De Pauw R Noten S, Meeus M.

Oral Presentation. IFOMPT Conference in Glasgow, Scotland. Altered grey matter morphology of pain processing regions in traumatic and non-traumatic neck pain patients: relations with clinical pain measures? **Coppieters I**, Meeus M, De Pauw R, Caeyenberghs K, Danneels L, Cagnie B. July 2015.

Poster presentation at the European Congress of Epidemiology – Healthy Living - Symposium Onderzoek in Beweging 'The Spine back on track' in Maastricht, The Netherlands. Symposium Onderzoek in Beweging 'The Spine back on track'. Effects of cognitive stress and relaxation on central pain modulation in patients with chronic whiplash-associated disorders, fibromyalgia and healthy controls. **Coppieters I**, Cagnie B, Van Oosterwijck J, De Pauw R, Meeus M. June 27 2015.

Poster presentation at the 9th Congress of the European Pain Federation EFIC in Vienna, Austria. Central pain modulation and cognitive functioning in patients with traumatic and non-traumatic chronic neck pain: preliminary results. **Coppieters I**, Cagnie B, De Pauw R, Danneels L, Ickmans K, Meeus M. 3 Sep 2015.

Poster presentation at Manual therapy congress in Louvain, Belgium. Altered grey matter morphology of pain processing regions in chronic whiplash and chronic idiopathic neck pain patients. **Coppieters I,** De Pauw R, Caeyenberghs K, Cagnie B, Danneels L, Meeus M. 18-19 Sep 2015.

Poster presentation at Manual therapy congress in Louvain, Belgium. Changes in brain morphology in patients with traumatic and non-traumatic neck pain. De Pauw R, **Coppieters I**, Caeyenberghs K, Danneels L, Meeus M, Cagnie B. Sep 2015.
Oral presentation at the Pain Science in Motion Congress, March 2017, Stockholm. Structural brain alterations in women with chronic whiplash associated disorders: relationships with cognitive deficits and disturbed pain processing. **Coppieters I**, De Pauw R, Caeyenberghs K, Danneels L, Kregel J, Meeus M*, Cagnie B*. *Equally contributed last authors.

Poster presentation at the Pain Science in Motion Congress, March 2017, Stockholm. Relations between brain alterations and clinical pain measures in chronic musculoskeletal pain: A systematic review. **Coppieters I**, Meeus M, Kregel J, Caeyenberghs K, De Pauw R, Goubert D, Cagnie B.

National Presentations

Verschillen tussen traumatische en niet-traumatische chronische nekpijn: de rol van centrale pijnverwerking, cognitief functioneren en structurele hersenveranderingen. Invited lecture voor het Lichtpuntje, vereniging van chronische pijnpatiënten en stichtend lid van de Vlaamse Pijnliga. Melle, Belgium. 21 May 2016.

Are there differences between traumatic and non-traumatic chronic neck pain patients? From science to practice. Invited lecture at a meeting of the Manual therapy Association (Mathera), Belgium, May 25th 2015, Vilvoorde, Belgium.

Differences between traumatic and non-traumatic chronic neck pain patients: the role of cognitive functioning, central pain modulation and structural brain changes. Invited lecture at a Belgian Back Society scientific meeting, December 1st 2015, Ghent, Belgium.

Oral presentation at the Research Day University Ghent, Faculty of Medicine and Health Sciences. Altered grey matter morphology of pain processing brain regions in patients with traumatic and non-traumatic chronic neck pain. March 2016.

Are there differences between traumatic and non-traumatic chronic neck pain patients? From science to practice. Iris Coppieters. Invited lecture at a meeting of the Manual therapy Association (Mathera), Belgium, May 25th 2015, Vilvoorde, Belgium.

Iris Coppieters. LOKK Peer review. 20 September 2016. Recente inzichten in de behandeling van patiënten met chronische pijn. Adinkerke. De Panne.

Acknowledgements - Dankwoord

Acknowledgements | Dankwoord

"*Gratitude is the memory of the heart.*" – Jean-Baptiste Massieu

Het maken van dit doctoraat was een zeer waardevol leerproces. De weg die ik de afgelopen 4 jaar heb afgelegd om tot dit eindresultaat te komen heeft mij veel voldoening gegeven en heb ik met veel passie en motivatie bewandeld. De tijd is vliegensvlug voorbijgegaan en mijn doctoraatsproject heeft mij verrijkt op professioneel en persoonlijk vlak. Dit doctoraat was niet mogelijk geweest zonder de enorme hulp en steun van heel veel mensen die mij omringen en die samen de onmisbare schakel vormden voor dit werk. Het dankwoord geeft mij de gelegenheid om iedereen te bedanken die mij heeft gesteund en heeft bijgedragen aan de totstandkoming van dit doctoraat.

Allereerst wil ik mijn promotoren oprecht bedanken: Prof. dr. Mira Meeus en Prof. dr. Barbara Cagnie. Ik ben jullie ontzettend dankbaar dat jullie mij de kans hebben gegeven om te doctoreren. Bedankt voor jullie vertrouwen en veel dank om steeds in mij te geloven. Van bij het begin kon ik altijd op jullie rekenen en hebben jullie mij enorm gestimuleerd om het beste uit mezelf naar boven te halen. Dankzij jullie indrukwekkende kennis, gedrevenheid en fantastische en complementaire begeleiding is dit doctoraat het afgewerkt geheel dat het nu is. Razendsnel hebben jullie mij steeds van waardevolle en constructieve feedback voorzien, waarvoor mijn oprechte dank.

Beste Mira, via alle media stond jij altijd voor mij klaar. Jouw steun en begeleiding hebben zeer veel voor mij betekend en gaven mij steeds de kracht om voor een nieuwe uitdaging te gaan en mijn grenzen te verleggen. Ik bewonder wat jij reeds bereikt hebt op jonge leeftijd.

Beste Barbara, je verbaast mij steeds opnieuw met je ongelofelijke efficiëntie en directe aanpak. Ik heb enorm veel van jou geleerd. Je bent een echte power woman! Bedankt om op zeer constructieve wijze mijn vaak veel te lange teksten grondig in te korten. Ondanks jouw drukke agenda stond jouw deur altijd open en heb je mij steeds met veel enthousiasme verder geholpen.

Mira en Barbara: ik hoop dat we nog lang kunnen blijven samenwerken en ben zeer verheugd dat we reeds aan een nieuw project, dat mooi aansluit op dit doctoraat, samen zijn begonnen. Ook bedankt voor de leuke momenten buiten het werk. Jullie zijn het beste promotorenteam dat ik mij kon wensen!

Veel dank gaat uit naar de leden van mijn begeleidingscommissie: Prof. dr. Karen Caeyenberghs en Prof. dr. Lieven Danneels, bedankt voor jullie waardevolle feedback, aanvullende kennis en begeleiding tijdens mijn doctoraatstraject. Karen, bedankt om mij als eerste wegwijs te maken in de boeiende wereld van MRI en het brein. Een oprechte dank voor de MRI tutorials die je ons gegeven hebt en voor de input bij de MRI papers.

Dear members of the examination board, I would like to express my sincere gratitude for your effort and time to read and evaluate my dissertation. Thank you for your interesting questions, valuable reflections on my work and good suggestions for improvement. I grateful acknowledge Prof. dr. Birgit Castelein, Dr. Kristine Oostra, dr. Inge Timmers, Prof. dr. Willem De Hertogh, dr. Wendy Scholten-Peeters, and dr. Roma Siugzdaite.

Ook wil ik Prof. dr. Patrick Calders hartelijk bedanken om als voorzitter de doctoraatsverdediging te leiden.

Verder wens ik alle personen te bedanken die hebben deelgenomen aan de onderzoeken. Zonder jullie deelname en inzet waren de studies van dit doctoraat niet mogelijk geweest. Ook een welgemeende dank u wel aan alle coauteurs die hun bijdrage hebben geleverd aan de publicaties.

To all the members of our Pain in Motion International Research Group, thank you for the interesting meetings, talks, fun weekends, and congresses. Thank you for all the support.

Ook gaan bijzondere woorden van dank uit naar mijn MRI collega's Helena, Hannelore en Mireille. Onze MRI meetings en brainstormsessies waren onmisbaar. De momenten die we samen hebben gedeeld buiten het werk waardeer ik enorm. Jullie aanmoedigingen waren een grote steun.

Een heel bijzondere dank gaat uit naar de collega's waarmee ik een bureau heb gedeeld tijdens de voorbije 4 jaar. Jullie waren mijn rotsen in de branding en een enorme steun tijdens iedere dag van mijn doctoraatsparcours. Ontelbare en leuke onvergetelijke momenten hebben we samen gedeeld zowel tijdens de werkuren als daarbuiten. Dankzij jullie was elke werkdag een plezier! Robby en Jeroen, ik ben heel dankbaar voor de sterke samenwerking die we al die jaren hebben gehad bij de urenlange testen, het analyseren van de MRI data en de late maar leuke scanuren op B3. Ook bedankt om mij te leren relativeren. Kayleigh en Anneleen, bedankt voor jullie onmisbare steun, vrolijkheid, positieve ingesteldheid en bruisend enthousiasme. Lieve bureaugenootjes, zonder jullie hulp, motivatie, vertrouwen in mij en vriendschap was dit doctoraat niet op deze manier gelukt! Met veel plezier denk ik terug aan onze congressen samen en de vele babbels en lachbuien tussendoor. Ik hoop van harte dat onze wegen elkaar zullen blijven kruisen, maar twijfel daar niet aan.

Verder wens ik alle REVAKI collega's te bedanken voor hun hulp, de leuke gesprekken, het enthousiasme en de fijne sfeer in de vakgroep. Een bijzondere dank gaat uit naar Dorien, Kelly, Evi, Stijn, Jessica, Tanneke, Birgit, Linda, Joke, Ruth, Bieke, Cedric, Vincent, Steven, Tom, Dorine, Dorien, Evy en Tine voor de leuke tijd samen.

Acknowledgements | Dankwoord

Buitengewoon veel dank gaat uit naar mijn familie en vrienden voor hun steun, interesse, goede raad en de leuke afleiding van mijn werk. Dank jullie wel voor alle gezellige momenten samen! Ik weet dat ik altijd op jullie kan rekenen. Bedankt om er voor mij te zijn. Een bijzondere dank u wel voor mijn oma en opa, jullie mogen niet ontbreken in dit dankwoord. Jullie waren altijd zo fier als een gieter als ik een goede school- of studieprestatie had neergezet en dat zou nu niet anders zijn geweest.

Ontzettend veel dank gaat uit naar mijn schoonouders Marleen en Francis, en lieve moeke Sonja en lieve peter Jef, bedankt om mij met heel veel liefde en warmte mee op te nemen in jullie hechte familie. De afgelopen 6 jaar stonden jullie steeds met open armen en met heel veel vreugde, inzet en plezier voor mij klaar. Jullie motivatie en wekelijks bezoek was een geweldige steun tijdens het tot stand komen van dit doctoraat.

Mijn bijzondere dank gaat uit naar mijn mama en papa. Bedankt voor jullie onvoorwaardelijke steun en liefde. Dank jullie wel om altijd in mij te geloven en mij aan te moedigen bij alles wat er reeds op mijn pad is gekomen. Ook bedankt voor alle kansen die jullie mij gegeven hebben en om altijd voor mij klaar te staan. Bedankt om mij een heel liefdevolle thuis te geven. Bedankt voor alles!

Graag richt ik een heel belangrijk woord van dank aan Ester, mijn dierbare zus en beste vriendin. Bedankt om altijd in mij te geloven en om te zijn wie je bent. Ik koester onze talrijke leuke zus-momenten die we samen reeds beleefd hebben. Altijd zijn we twee handen op één buik geweest en dit heeft mij steeds opnieuw veel energie gegeven.

Jeffrey, lieve schat, levenspartner, mijn steunpilaar en soulmate. Wat ben ik het lot dankbaar dat onze wegen elkaar gekruist hebben. Bedankt voor je onvoorwaardelijke liefde, vertrouwen in mijn kunnen, jouw buitengewone intelligentie, humor en levenswijsheid. Jouw steun is onmisbaar. Je kent me beter dan eender wie. Bedankt om een warme thuis te voorzien, een plek waar ik volledig mezelf kan zijn. Ik kijk uit naar alle mooie momenten die we nog samen zullen delen tijdens ons levensavontuur. Jij maakt mij gelukkig!

Iris Coppieters
Gent, mei 2017